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Rapid Photometric Methods for Determining Rubber and Resins in Guayule Tissue and Rubber in Crude-Rubber Products[1]

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INTRODUCTION

In order to achieve the maximum progress in research on the physiology of guayule (*Parthenium argentatum* A. Gray) it is imperative

[1] Submitted for publication January 3, 1946.

[2] Credit is due Beatrice K. Dewald, main scientific aide, for assistance in the laboratory.

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to have rapid methods for the determination of rubber and "resins" [3] as well as the already available rapid analytical procedures for the other plant constituents. In this bulletin are described new, rapid photometric methods for the analysis of rubber and resins in small samples of guayule and of rubber in tissues of other plants and in crude-rubber products.

The classical method of Spence and Caldwell (*5*)[4] is commonly used for the determination of rubber and resins in plant tissue. Duplicate 5-gm. samples are used. Water solubles are first removed by boiling in 1-percent sulfuric acid for 3 hours; the extract is then autoclaved for 3 hours and leached for 3 hours. The samples are next extracted in a reflux-type apparatus with acetone for 12 hours to obtain the acetone extract; finally they are extracted with benzene in the same type of apparatus for at least 16 hours. After 5 ml. of a 0.1-percent solution of dimethyl-*p*-phenylenediamine has been added to the benzene extract, the benzene is evaporated and the rubber is weighed.

Employees of the Intercontinental Rubber Co. [5] and of the Eastern Research Laboratory, Bureau of Agricultural and Industrial Chemistry,[6] and Holmes and Haasis [7] have used modified Spence-Caldwell procedures.

According to the Holmes-Haasis modification, which is used in certain comparisons discussed in this bulletin, the size of the sample is reduced to 3 to 4 gm.; water solubles are extracted by 2-percent (instead of 1-percent) sulfuric acid for 1½ hours (instead of 3); the subsequent autoclaving is for 1½ hours (instead of 3) and the leaching for 1 hour (instead of 3). Samples are then extracted for 8 hours (instead of 12) with acetone in a reflux-type apparatus to obtain the acetone extract; they are finally extracted in the same type of apparatus for 16 hours with benzene. After 1 ml. of a 0.6-percent solution of phenyl-α-naphthylamine (instead of 5 ml. of a 0.1-percent solution of dimethyl-*p*-phenylenediamine) has been added to the benzene extract, the benzene is evaporated and the rubber weighed. This modified Spence-Caldwell method is referred to in the comparisons with the present method.[8]

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[3] The term "resins" as used herein refers to the portion of the acetone or acetonyl-acetone-cellosolve 3–7 extract of guayule tissue that is precipitated by 0.5-percent H_2SO_4 in H_2O. This precipitate represents a mixture of several substances. An inventory of what is known about this fraction is given on page 29. From a physiological standpoint this fraction is considered at present as an approximate measure of the contents of the "resin" canals and any "resins" that may be included in the cells. The word "resins" without quotation marks is used in the text in this sense.

[4] Italic numbers in parentheses refer to Literature Cited, p. 37.

[5] Information in the files of the Salinas division, Intercontinental Rubber Co., now the property of the U. S. Department of Agriculture.

[6] ANALYTICAL SECTION, EASTERN REGIONAL LABORATORY. PROCEDURES FOR THE ANALYSIS OF RUBBER-BEARING PLANTS AND THEIR DERIVED PRODUCTS. [Unpublished.]

[7] HOLMES, R. L., and HAASIS, F. W. NOTES ON THE SPENCE-CALDWELL METHOD OF RUBBER ANALYSIS. Bur. Plant Indus., Soils, and Agr. Engin., Spec. Guayule Res. Proj. Rpt. [Unpublished.]

[8] Credit is due R. L. Holmes, F. W. Haasis, and their assistants in the rubber laboratory, Special Guayule Research Project, Salinas, Calif., for making the rubber and resins determinations by the modified Spence-Caldwell method for use in the comparisons recorded.

Cox [9] proposed a turbidimetric method for rubber analysis. According to this procedure, 5 gm. of ground material is extracted with 100 ml. of benzene for 15 minutes in a Waring blender or with constant shaking; the supernatant liquid is clarified by centrifuging and is made up to a 100-ml. volume. The rubber in 5-ml. aliquots is precipitated by the addition of 1 ml. of carbon tetrachloride plus 20 ml. of acetone. Rubber is determined turbidimetrically on aliquots of the extract in a Fisher 155-volt a. c. model electrophotometer fitted with a 525 B green filter. Jones and Wildman [10] proposed a modified form of the Cox method: 0.3-gm. samples of the ground material are extracted overnight with 10 ml. of benzene; the extract is clarified by centrifuging and decanted into a 25-ml. volumetric flask, and the residue is extracted three additional times with 5-ml. portions of benzene; in each case centrifuging and decanting of the liquid, which is added to the original extract, follow. After the extract is made up to a 25-ml. volume, 3-ml. aliquots are pipetted into 5 ml. of acetone in test-tube cuvettes; the resulting rubber suspension is mixed, and the amount of rubber is determined by the reading of scattered light on a Coleman electronic photofluorometer.

The above-mentioned procedures for rubber analysis were tested in the writer's laboratory but did not give precise and accurate results apparently because of the nature of the rubber solvents and precipitants used, the errors caused by extraneous color in the extracts, and the failure to control sufficiently the change in the photometric readings of rubber in colloidal solution on standing.

Apparently no photometric method for resins analysis in plant tissue has been reported.

The present photometric methods for analysis of rubber and resins are based on the following principles: (1) Grinding the properly dried tissues to the required degree of fineness to permit quantitative extraction of rubber or resins; (2) using oxygenated solvents with relatively high boiling points (115.8° to 230.7° C.), alone or in mixture, that permit the rapid extraction of rubber or resins in 20 to 30 minutes at relatively high temperatures (115° to 120°); (3) precipitating the rubber or resins from aliquots of the solutions by means of suitable precipitants; (4) making photometric readings of percentage of light transmittance after rubber or resins precipitation at a wave length that eliminates the error due to extraneous color (the determination relying on the development of a uniform turbidity (*7, pp. 1–2*) rather than a color for purposes of analysis); (5) securing this uniform turbidity, or required degree of precision, by making the photometric readings at standing times that are based on the concentration of rubber or resins precipitated. (The relation of concentration of precipitated rubber or resins to stability of photometric readings is developed in detail on pages 24 to 25.)

In rubber analysis the use of the oxygenated solvents with higher boiling points eliminates the use of the highly volatile and toxic ben-

[9] Cox, L. C. A TURBIDIMETRIC METHOD FOR THE ANALYSIS OF PLANT MATERIALS FOR RUBBER. Rubber Reserve Corp Rpt. [Unpublished.]

[10] Jones, E. J., and Wildman, S. G. A RAPID, SEMI-MICRO METHOD FOR THE ANALYSIS OF RUBBER IN PLANT MATERIAL. Bur. Plant Indus., Soils, and Agr. Engin., Div. Rubber Plant Invest. Rpt. [Unpublished.]

zene. The solvent, methyl isobutyl ketone, on which most of the experimental work with the rubber method is based, is reported as nontoxic (*9*).

In physiological experiments, the amount of available material is often very low and rubber percentages below 1 percent are not uncommon. Hence the need for methods equally accurate for all ranges of rubber contents and requiring a minimum of plant material is evident. It is believed that the methods described on pages 4 to 16 meet these specifications. In addition, the procedures described there are relatively rapid.

Although these methods were developed primarily for use in research on guayule physiology they have wider application. They have been used in routine guayule rubber and resins analysis on an equal basis with the modified Spence-Caldwell method in the general rubber laboratory, Special Guayule Research Project, since August 1943. They should also be of value in determining rubber and resins in guayule breeding material and in other studies for which plant material is limited

PROCEDURES

Preparation of Samples

The details of preparation of the tissue samples are kept constant throughout any particular research project. When portions of the same samples are taken for chemical analyses besides rubber and resins, the plant parts are dried without pretreatment. Whether separate samples are taken for the other analyses or the sole purpose is obtaining an estimate of the total rubber and resins, the plants, or plant parts are placed in boiling water for 8 minutes (parboiled),[11] and any leaves still adhering to the stems are removed by tapping on a table top.

The plants or plant parts are subsequently coarsely ground in a Wiley or other suitable mill and dried to 5 percent or less moisture in a mechanical convection oven at 65° C. before the final grinding. A drying period of at least 24 hours is required. The dried tissue is then ground in a hammer mill (*4*) until it passes through a 2- or 2.5-mm. screen. The mill is provided with a ground wire to remove static electricity from the sample. Between samples it is cleaned thoroughly by blowing with compressed air.

The ground samples are stored in airtight containers. Wide-mouth bottles with metal or plastic screw tops are most convenient for this purpose.

Since the results in most physiological experiments are expressed on the basis of the dry weight of the original sample dried to constant weight at 100° C., it is desirable to express the final rubber and resins contents on the same basis. A correction factor is therefore determined on the finely ground, thoroughly mixed sample by drying small duplicate subsamples (1 to 2 gm.) for 8 hours at 100° in an ordinary

[11] The practice of parboiling guayule shrub for removal of leaves was developed by employees of the Intercontinental Rubber Co. (L. M. Caldwell, Experiment S–249, Sept. 15, 1928) and also applied by other employees in later years. This was ascertained from the files of the Intercontinental Rubber Co., now the property of the U. S. Department of Agriculture.

drying oven. When the samples are very small, minimum amounts of tissue in very small weighing bottles (3.5 cm. high and 2.2 cm. in diameter) are used to conserve tissue. The samples for analysis are then weighed out on the basis of constant dry weight at 100° by using the factor for moisture correction.

Reagents for Rubber Analysis

In a number of cases in this section and the one on Reagents for Resins Analysis technical-grade chemicals, which were the best obtainable during the war period, are indicated. These proved adequate, but when better grades are available they can be checked to determine any advantages of substituting c. p. grades. The rubber and resins solvents are kept in glass-stoppered bottles.

Solvents.—Methyl isobutyl ketone, technical-grade, Carbon & Carbide Chemicals Corp., is entirely satisfactory for natural rubber. It is reported as apparently nontoxic (*9*). Although it has the characteristic ketone odor, this is less pronounced than that of other ketones. Constants for this and other rubber solvents are given in table 1.

TABLE 1.—*Properties of selected rubber and resins solvents completely soluble in 95-percent ethanol*

Solvent	Formula	Boiling point (760 mm. Hg)	Flash point	Solubility of unvulcanized rubber	Solubility (relation to weight at 20° C.)	
					Solvent in water	Water in solvent
Rubber solvents:		°C.	°C.		Pct.	Pct.
Benzene [1]	C_6H_6	80.1	11.0	Soluble	0.08	-------
Methyl isobutyl ketone.	$(CH_3)_2CHCH_2COCH_3$	115.8	23.9	do	.2	2.2
Methyln-amyl ketone	$CH_3CH_2CH_2CH_2CH_2COCH_3$	150.6	48.9	do	.43	1.5
Diisobutyl ketone	$(CH_3)_2CHCH_3COCH_2CH(CH_3)_2$	168.1	60.0	do	.06	.45
Butyl acetate	$CH_3COOC_4H_9$	126.3	37.8	do	.68	1.2
Methyl amyl acetate	$CH_3COOCH(CH_3)CH_2CH(CH_3)_2$	146.3	43.3	do	.13	.58
Ethylene glycol diethyl ether.	$C_2H_5OCH_2CH_2OC_2H_5$	121.4	35.0	do	21.0	3.4
N-butyl ether	$C_4H_9OC_4H_9$	142.2	37.8	do	.03	.19
Resins solvents:						
Acetone [1]	CH_3COCH_3	56.1	−9.4	Insoluble	100	100
Acetonylacetone	$CH_3COCH_2CH_2COCH_3$	191.4	85.0	do	100	100
Ethylene glycol monoethyl ether (cellosolve).	$C_2H_5OCH_2CH_2OH$	135.1	57.2	do	100	100
Diethylene glycol monobutyl ether (butyl "carbitol").	$C_4H_9OCH_2CH_2OCH_2OH$	230.7	115.6	do	100	100

[1] Included for comparison only.

Rubber-precipitating solution.—A 0.5-percent H_2SO_4 (c. p. reagent, Du Pont, sp. gr. 1.84) solution in 95-percent ethanol by volume is used as a rubber precipitant.

Protective Colloid Solution for Rubber.—A 4.5-percent solution of diglycol stearate S or glycosterin (a refined grade of diglycol stearate S not available during the war period) (both technical-grade, Glyco Products Co.[12]) is made in morpholine (technical-grade, Eastman). (The stock of diglycol stearate S used in these experiments, on the basis of three determinations, at 25° C. was 5.11±0.047 percent soluble in morpholine.) The diglycol stearate S is brought into solution by heating; the solution is made up to volume at a standard temperature (25°

[12] 230 King Street, Brooklyn, N. Y.

in the writer's laboratory) and filtered. A 5-percent solution of this by volume is then made in the ketone, acetate, or ether, or the mixture of these used as the rubber solvent.

Reagents for Resins Analysis

Resins solvents.—A mixture of 3 parts of acetonylacetone and 7 parts of ethylene glycol monoethyl ether (hereinafter referred to as cellosolve) by volume (both technical-grade, Carbon & Carbide Chemicals Corp.) is a good resins solvent. This mixture is referred to as acetonylacetone-cellosolve 3–7 in the text. Constants of these and other resins solvents are given in table 1.

Resins-precipitating solution.—A 0.5-percent H_2SO_4 (c. p. reagent, Du Pont, sp. gr. 1.84) solution in H_2O by volume is used as the resins precipitant.

Protective Colloid Solution for Resins.—A 0.2-percent solution of diglycol stearate S or glycosterin is made in acetonylacetone-cellosolve 3–7 or any other resins solvent used. The diglycol stearate S is dissolved by heating; the solution is made up to volume at a standard temperature and filtered. If the solution should become cloudy because of a drop in temperature, the precipitate may be brought back into solution by heating.

Special Equipment for Rubber and Resins Analysis

PHOTOMETRIC INSTRUMENTS

Several types of instruments, including the Coleman electronic photofluorometer, Coleman double monochromator spectrophotometer, Klett-Summerson colorimeter, and Evelyn photoelectric colorimeter, have been used. The photofluorometer apparently is not suited for the purpose because the characteristic of the suspension measured was such that there was very rapid change in the readings on standing of the solution after rubber and resins precipitation. The order of preference from the standpoint of greatest precision of readings is as follows:

(1) Coleman double monochromator spectrophotometer model 10S or Coleman universal spectrophotometer model 11.—Wave-length setting at 750 mμ is recommended.

(2) Evelyn photoelectric colorimeter with Corning monochromatic filter combination 720 mμ (2432:3952:5852).—A plastic cap is used to cover absorption test tube when readings are being made. A rack for holding a hand magnifying glass to facilitate making approximate galvanometer readings to 0.1 may be manufactured locally.

(3) Klett-Summerson colorimeter.—No suitable filter combination of approximately 750 mμ wave length is available at present. The instrument performs satisfactorily with the available filters between 575 and 675 mμ for rubber analysis on 2-year-old or older guayule material with leaves removed and for current-season growth from such plants with filters above 450 mμ (see fig. 9). This limits its usefulness.

For the present the Evelyn photoelectric colorimeter is recommended as the most convenient and adaptable instrument for routine laboratory analyses.

OTHER EQUIPMENT

A thermostatically controlled oil bath with a removable screen basket partitioned to hold 25-ml. volumetric flasks (fig. 1). High-grade cylinder oil S. A. E. 20 is used.

A thermostatically controlled water bath provided with motor stirrer and removable screen basket partitioned to hold 25- and 100-ml. volumetric flasks for use in standardizing temperature of reagents and aliquots of extracted samples taken for analysis.

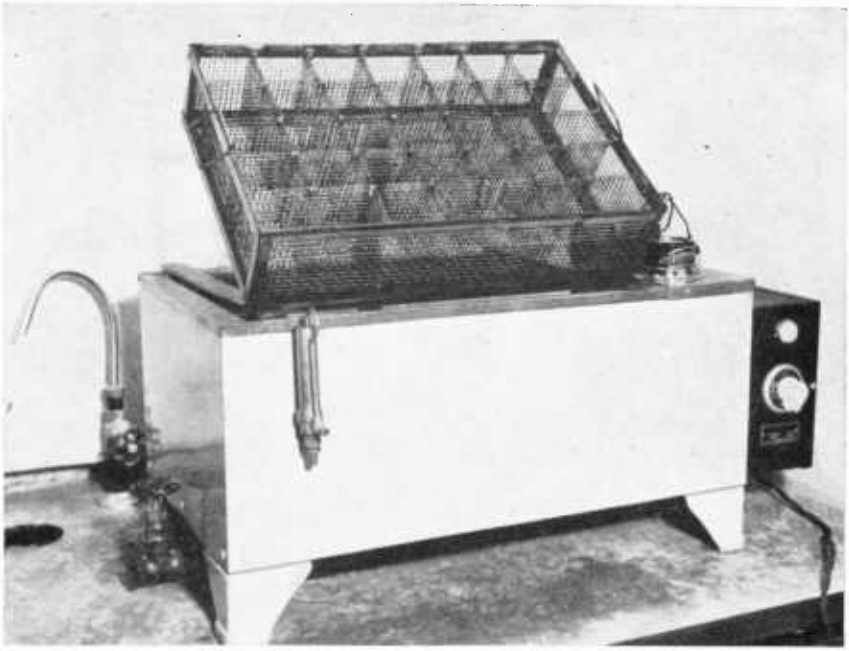

FIGURE 1.—American Instrument Co. constant-temperature, general-purpose bath used as an oil bath. Note special removable wire basket for holding 25-ml. volumetric flasks during extraction of rubber and resins; this is not standard equipment and must be added by the analyst.

Model E measured time clock (Measured Time, Inc.) and Kodak electric timer; the latter was used in determining standing time accurately to the minute after rubber and resins are precipitated and the former for timing heating and centrifuging periods.

A Wiley or a Ball & Jewell mill for the initial coarse grinding of guayule tissue.

Ross-Hardesty modification of the hammer mill (*4*) provided with No. 1 (2-mm.) and No. 2 (2.5-mm.) screens.

Pyrex 25- and 100-ml. volumetric flasks provided with ground-glass stoppers.

A special 30-ml. Pyrex trituration beaker with flat inside bottom and glass rod, 12 cm. long by 0.5 cm. in diameter, with ground flattened end 1 cm. in diameter, for use in making rubber solutions (fig. 2, *A* and *B*). The special beaker is manufactured from the 30-ml. Griffin low-form beaker with spout (Corning Glass Works No. 1000). The bottom is flattened by the regular glass-working procedure. The flattened end of the glass rod and the surface of the flat inside bottom of the beaker are ground together with emery in order to secure the most satisfactory surface for trituration.

A Pyrex watch glass, at least 2 mm. in thickness, for use as a base in dividing rubber samples.

A cork knife for use in dividing rubber samples.

Heavy-duty Pyrex 40-ml. graduated centrifuge tubes provided with cone points and cork stoppers to fit (fig. 2, *C*).

Filter sticks (fig. 2, *D*) for use with the graduated tubes in the operation of pouring clarified extracts into 25-ml. Erlenmeyer flasks without disturbing residue. At the outset a glass rod with a flattened end was manufactured in the laboratory for this use, but it was difficult to secure a perfect fit. However, the available filter stick served the purpose and was later substituted. The fritted glass disk serves no function as such in this operation but holds back the liquid and residue in the cone point.

Caulfield pipette for transferring ketones and other organic solvents.

Small weighing bottles 3.5 cm. high and 2.2 cm. in diameter.

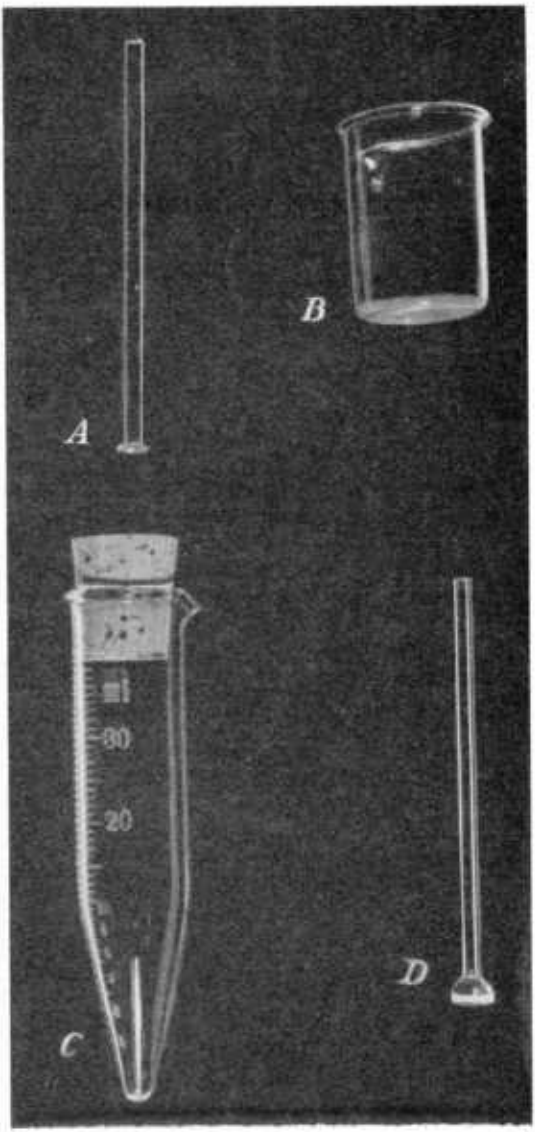

FIGURE 2.—Some of the special glassware required for rubber and resins analysis: Glass rod with flattened end (*A*) and trituration beaker (*B*) for use in making stock solutions of rubber; corked, heavy-duty, 40-ml. Pyrex centrifuge tube with cone point (*C*); and filter stick (*D*) for use in pouring off clarified extracts without disturbing the insoluble residue.

Glass funnels, 1 inch in diameter, for use in transferring weighed samples and clarified extracts to 25-ml. volumetric flasks.

Wide-mouth, glass bottles with plastic or metal screw caps for storing ground plant samples.

Racks for holding 50-ml. centrifuge tubes and 25-ml. volumetric flasks (fig. 3).

Automatic 100-ml. Pyrex burette, of the type used for acids, with interchangeable ground joint, for rapid delivering of rubber and resins precipitants.

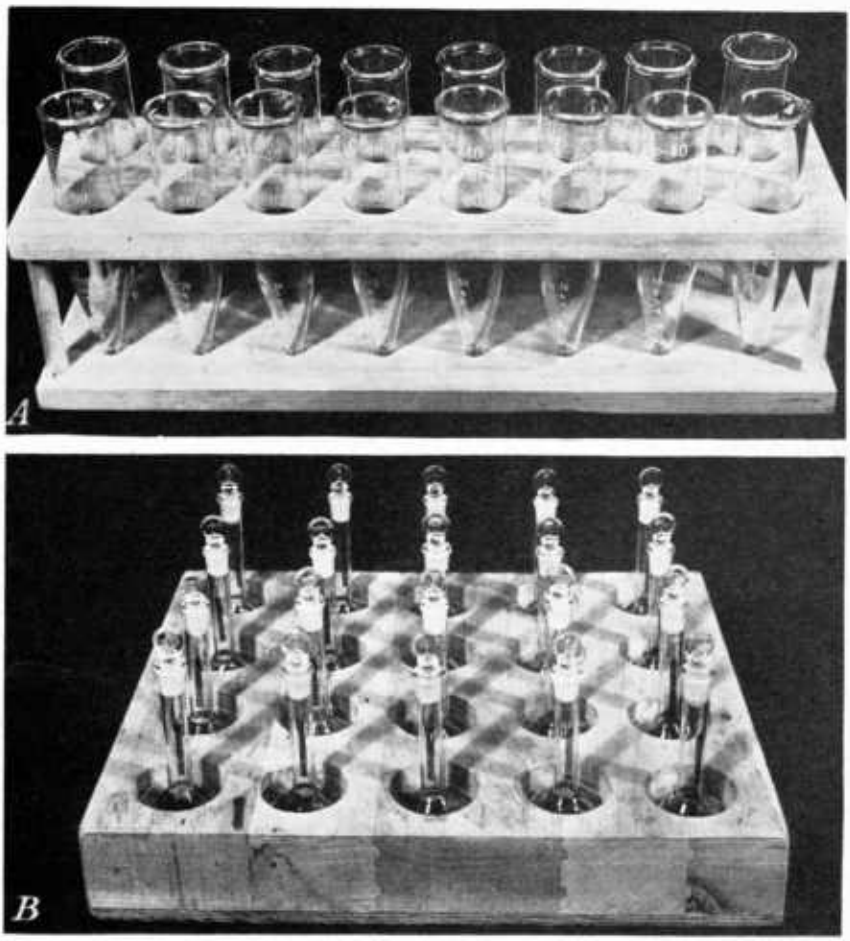

FIGURE 3.—Racks required for holding (*A*) centrifuge tubes and (*B*) 25-ml. volumetric flasks.

CLEANING GLASSWARE

The importance of a high degree of cleanliness in photometric work is discussed by Yoe (*8, pp. 33–34*). Centrifuge tubes are first scrubbed thoroughly with soap and tap water, and then they are rinsed with cleaning solution and water. If flasks with precipitated rubber or resins are to stand any length of time before they are washed, it is advisable to pour out the contents and replace with tap water. The

25- and 100-ml. volumetric flasks are rinsed two or three times with tap water and then with cleaning solution and water as is customary. In all cases the rinsed glassware is dried in an inverted position at room temperature or upright in a drying oven. The flasks used for extraction of rubber from kok-saghyz (*Taraxacum kok-saghyz* Rodin) are finally rinsed with the solvent to be used and are allowed to drain and dry under a ventilated hood. Cuvettes or absorption test tubes are handled and cleaned as directed by the manufacturers of the photometric instruments used.

Rubber (Caoutchouc) Calibration Curves

GENERAL CONSIDERATIONS

The rubber hydrocarbon standard is prepared by extracting the rubber from guayule tissue according to the Spence-Caldwell method (*5*) or by the Holmes-Haasis modification of this method (p. 2). The air-dry extracted rubber is dried further in a vacuum oven with 30 inches of vacuum at 65° C. for 4 hours. The rubber standard is preserved in a closed container stored in a refrigerator. Before the samples are weighed out, the unopened container with the rubber is brought again to room temperature.

Two stock solutions, each in triplicate from separately extracted standard rubber samples, are prepared—the first set of three containing 0.1 percent, or 1 mg. of rubber per milliliter, and the other 0.2 percent, or 2 mg. of rubber per milliliter. The desired weight of rubber contained in a known volume of the solvent is withdrawn from a burette or pipetted and made up to 25 ml., the standard volume used throughout the procedure.

The rubber samples (0.1 and 0.2 gm.) for the stock solutions are weighed out to an accuracy of 0.2 mg. on an analytical balance. The rubber is best manipulated by means of the straight blunt-edged top of a cork knife. With a little practice one can readily separate the desired portions by pressure from above. Further subdivision is made on a stout Pyrex watch glass, at least 2 mm. in thickness. By pressure from above and shearing action very small particles can be separated so that the required amounts can be weighed out accurately. The rubber sample is then divided into small pieces on the special watch glass by means of a cork knife.

One milliliter of diisobutyl ketone [13] is placed in a special 30-ml. trituration beaker (fig. 2, *B*) and the finely divided rubber sample (0.1 or 0.2 gm.) is added and heated on a hot plate under a ventilated hood to a temperature just under the boiling point of the solvent. A special glass rod is used to triturate the sample (fig. 2, *A*).

When the rubber has dissolved so as to make a homogeneous gellike mass, hot methyl isobutyl ketone or any of the other rubber solvents recommended (table 1) is added and mixed with the rubber solution.

[13] Diisobutyl ketone was not available during wartime. Methyl isobutyl ketone may be substituted, but it is not as efficient in this operation. If the latter is used, it is best to let the finely divided rubber particles stand overnight in the solvent and to cover the container to minimize evaporation of solvent before heat is applied to complete solution.

The mixture is transferred to a 100-ml. volumetric flask with several washings of hot solvent, cooled to a standard temperature, and made up to volume with the same solvent. Special precaution must be taken in transferring the rubber solution to the volumetric flask so that no rubber is lost. It is convenient to insert a 4-inch funnel in the neck of the volumetric flask to pour the rubber solution in one operation into the tube of the funnel, with the aid of the glass rod, and to follow immediately with several washings of hot solvent, which are first poured into the beaker held directly over the funnel and then through the funnel.

If time is not an object, it is also feasible to place the finely divided particles of rubber in the flask containing the solvent and allow it to stand until the rubber is dissolved. The rubber solutions are stored in the tightly stoppered volumetric flasks in a cool, dark place. There they will remain constant in value for an indefinite period.

Two calibration curves are made—one for 0.2 gm. of tissue containing 0.1 to 1.0 percent of rubber (0.04 to 0.4 mg. of rubber in a 25-ml. volume) and the other for 0.2 gm. of tissue containing 1.0 to 25.0 percent of rubber (0.4 to 10 mg. of rubber in a 25-ml. volume).

CURVES FOR 0.1 TO 1 PERCENT OF RUBBER

In order to reduce error to a minimum, a suitable microburette is used to prepare the set of standard solutions of varying concentrations in the range 0.1 to 1 percent of rubber. In the present work the Improved Rehberg microburette (*1, 3*), which accurately delivers to 0.001 ml., was found satisfactory. From a 0.1-percent standard rubber solution in a suitable solvent quintuplicate aliquots of 0.04, 0.1, 0.2, 0.3, and 0.4 ml. are measured out at a standard solution temperature (25° C. in writer's laboratory) by means of a microburette into 25-ml. volumetric flasks provided with glass stoppers. Enough of the rubber solvent is then added to each flask to make 5 ml. when added to the amount of rubber solution previously added. After 0.1 ml. of protective colloid solution has been added the rubber is precipitated and spectrophotometer or colorimeter readings are made according to the procedure outlined on page 14. The readings for each concentration are averaged and plotted against the milligrams of rubber in a 25-ml. volume, or percentage of rubber calculated on the basis of 0.2-gm. tissue samples and aliquot volume factors for the procedure outlined on page 14. This procedure is repeated twice; in each case a standard rubber solution was prepared from separately extracted standard rubber samples. The complete data are plotted on coordinate paper as shown in figure 4.

CURVES FOR 1 TO 25 PERCENT OF RUBBER

The procedure in making a curve for 1 to 25 percent of rubber is similar to that for the curve for 0.1 to 1 percent, except in a few details. The quintuplicate aliquots are spaced at 0.4, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg. per 25-ml. volume. In securing the 0.4 mg. per 25-ml. volume, the procedure outlined in the preceding paragraph is followed. In the range 1 to 4 mg. per 25-ml. volume, the desired concentrations of rubber are secured by measuring aliquots with National Bureau of Standards transfer pipettes from the 0.1-percent rubber solution.

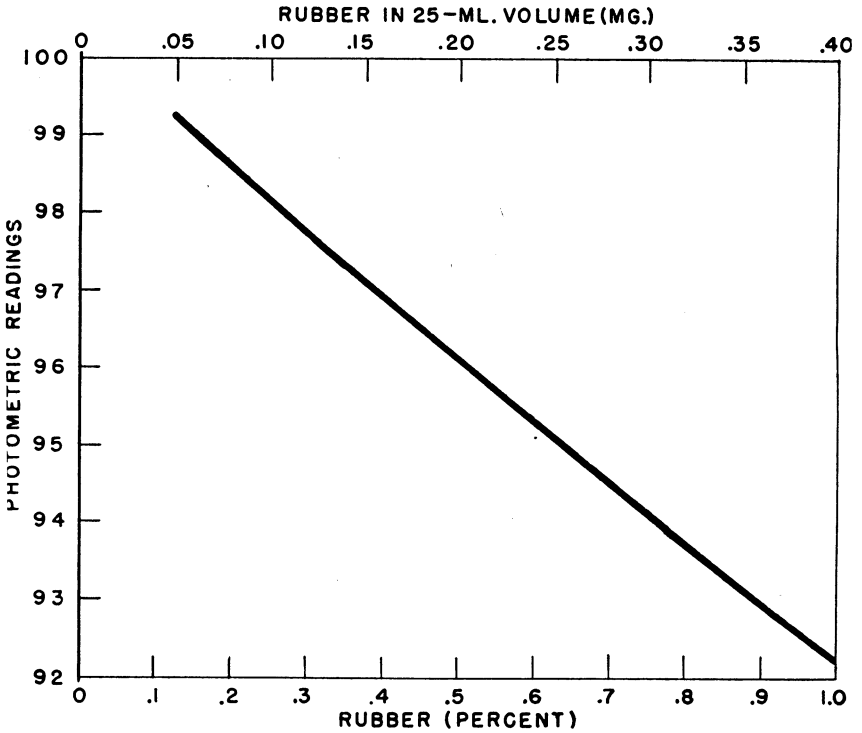

FIGURE 4.—Calibration curve for rubber in 0.2 gm. of guayule tissue containing 0.1 to 1.0 percent of rubber. (Readings on an Evelyn photoelectric colorimeter with 720-mμ filter and 6-ml. aperture; the 0.1 divisions required in actual practice for photometric readings are omitted.)

Similarly, in the range 5 to 10 mg. per 25-ml. volume, the desired concentrations of rubber are measured from the 0.2-percent solution. The complete data are plotted on semilogarithmic paper as shown in figure 5, *A*.

STANDARDIZATION AGAINST GRAVIMETRIC METHOD

The alternate procedure of standardizing the present method against the Spence-Caldwell method (*5*), although feasible, is not advisable because of the extra work required and the introduction of the experimental error of the benzene extraction method.

RESINS CALIBRATION CURVES

The procedure for constructing calibration curves for resins analysis is similar to that outlined for rubber, except that resins exudate of guayule is used as the resins standard. The resinous exudate dissolves very readily in the resins solvents listed in table 1. It may be prepared in two ways for use as a standard—the clean exudate as gathered from the plants is dried for 6 hours at 65° C. at a 30-inch vacuum or the exudate is dissolved in acetone and filtered. In drying

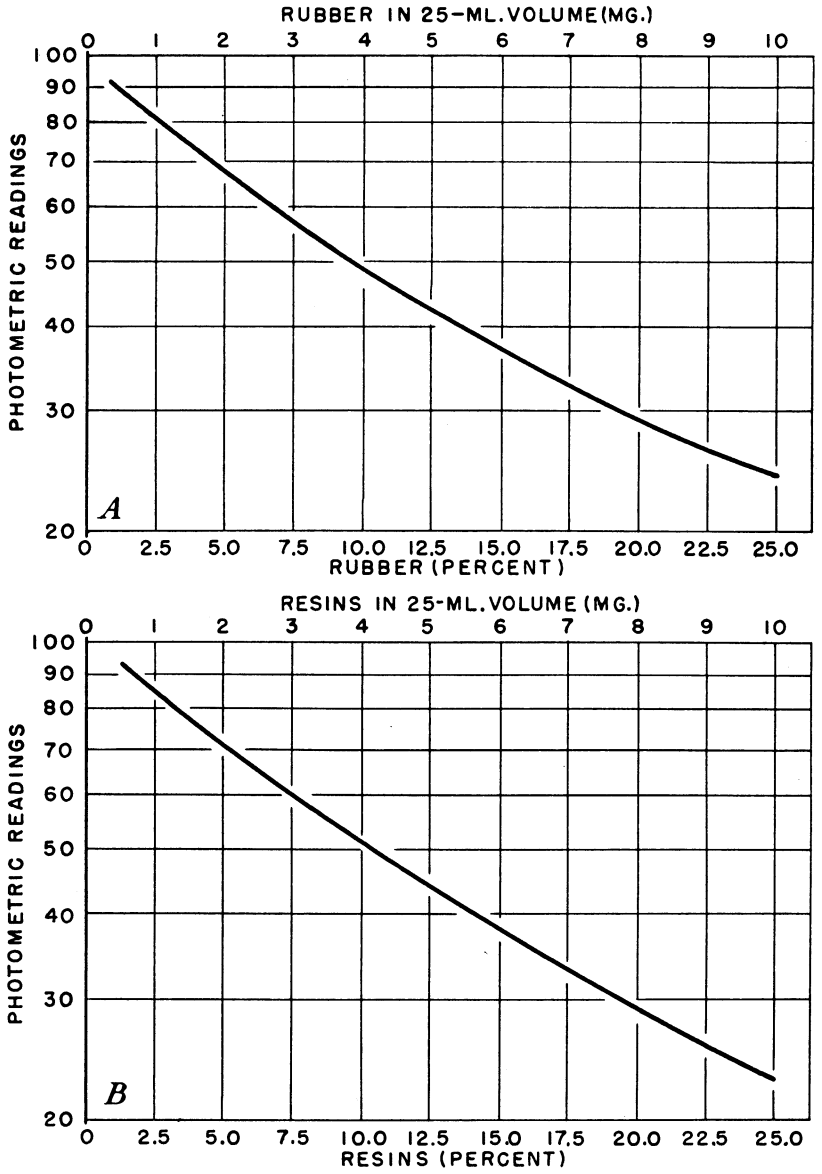

FIGURE 5.—Calibration curve for (*A*) rubber in 0.2 gm. of guayule tissue containing 1 to 25 percent of rubber and (*B*) resins in 0.2 gm. of guayule tissue containing 1 to 25 percent of resins. (Readings on an Evelyn photoelectric colorimeter with 720-mμ filter and 6-ml. aperture; the 1.0 divisions required in actual practice for photometric readings are omitted.)

in vacuo, a relatively small quantity of exudate is placed in a large dish, since the mass foams at the beginning of the drying process. Practically all of the acetone is evaporated off over a steam or water bath, and the residue is dried, pulverized in a mortar, and preserved by

drying as indicated for fresh exudate. In all cases the products are stored in airtight containers in a dark, cool place.

The usual range of resins from guayule tissue is about 2 to 8 percent on a dry-weight basis. For work with this plant, therefore, only one curve is needed. A typical resins calibration curve is shown in figure 5, *B*.

Procedure for Rubber Analysis

Duplicate extractions are made for each tissue analyzed and duplicate photometric readings are made for each extraction; the final value therefore reported represents an average based on the four photometric readings.

Thoroughly mixed 0.2-gm. samples of the finely ground tissue are weighed for analysis on the basis of moisture-free tissue. The samples are weighed to an accuracy of 0.2 mg. on an analytical balance. The method of deriving factors for making corrections for moisture in samples has been detailed on page 4.

The weighed samples are transferred to the 25-ml. volumetric flasks with the aid of 1-inch glass funnels, and 20 ml. of methyl isobutyl ketone is added to each flask. The contents in the flasks are heated in a constant-temperature oil bath at 115° C. for 30 minutes under a ventilated hood. The flasks are removed and any adhering oil on the outside is wiped off with Kleenex or a similar cellulose tissue; the contents of the flasks are cooled to a standard temperature (25° in the writer's laboratory) in a constant-temperature water bath provided with a motor stirrer, if available, and made up to volume with the solvent. After the contents are mixed, the flasks are allowed to stand for a few minutes until most of the insoluble residue settles to the bottom. The extracts are then carefully poured into 40-ml. heavy-duty centrifuge tubes so as to include as little as possible of the settled insoluble residue. The centrifuge tubes are stoppered with cork stoppers, and the liquid is clarified by centrifuging for 10 minutes at 2,000 revolutions per minute. The clarified liquid is poured into 25-ml. volumetric flasks provided with ground stoppers. *It is important to pour off the clarified extract in such a manner that the insoluble residue is not stirred up.* A 1-inch funnel is placed in the neck of the volumetric flask. A filter stick (fig. 2, *D*) is inserted in the centrifuge tube through the liquid so that the enlarged part of the stick seals off the insoluble residue and a small portion of the extract in the cone point. With the index finger the stem of the filter stick is then held against the side of the tube toward the analyst, who holds the tube with the thumb and third finger. The extract is then poured off easily into the volumetric flask without disturbing the insoluble residue. The contents of the flasks are then brought to the standard temperature in the constant-temperature water bath.

The reagent blank used with the spectrophotometer or colorimeter is prepared as follows: 5 ml. of methyl isobutyl ketone or other solvent used is pipetted into a 25-ml. volumetric flask and 0.1 ml. of protective colloid solution for rubber is added; the mixture is made up to volume with acidified 95-percent ethanol and mixed again; the required volume of the mixture is poured into a cuvette or an absorption test tube.

From each pair of tissue extractions, two 5-ml. aliquots of the clarified extract, at the standard temperature, are pipetted into 25-ml. volu-

metric flasks and 0.1 ml. of protective colloid solution for rubber is added to each. One additional 5-ml. aliquot from each pair of extractions is similarly prepared to serve as a key to the standing times for the final readings as indicated in table 2. These additional aliquots are first made up to volume with the precipitant, acidified 95-percent ethanol, mixed by carefully inverting the flask three or four times, and allowed to stand for 4-minute periods before the reading is made on the colorimeter. With these preliminary readings as a basis, the standing times for final readings are taken from table 2. For example, if the colorimeter reading in rubber analysis at 4 minutes is 65.5, then the standing time is 15 minutes.

The two remaining duplicate aliquots for each extraction are then made up to volume with acidified 95-percent ethanol, mixed by carefully inverting the flask three or four times, and allowed to stand for the required number of minutes, as established by the preliminary 4-minute trial readings (table 2), before the final colorimeter readings are made on the contents of the flasks.

TABLE 2.—*Standing time for various concentrations of rubber or resins after precipitation before colorimetric readings are made*

[5-ml. aliquots of rubber in methyl isobutyl ketone or of resins in acetonylacetone-cellosolve 3-7; both made up to a 25-ml. volume with precipitant; readings on Evelyn photoelectric colorimeter with 720-mμ (690 to 770-mμ) filter and 6-ml. aperture]

RUBBER [1]

Range of colorimeter trial readings after 4-minute standing time [2]	Approximate range in content in tissue	Standing time for final reading	Range of colorimeter trial readings after 4-minute standing time [2]	Approximate range in content in tissue	Standing time for final reading
	Percent	*Minutes*		*Percent*	*Minutes*
95.5–99.9	0.9–0.1	60	55.1–63.8	9.3–6.9	10
74.3–95.4	4.3–1.0	30	42.0–55.0	13.7–9.4	5
63.9–74.2	6.8–4.4	15	Below 42	More than 13.7	2

RESINS [3]

95.7–99.9	1.39–0.1	60	41.0–54.1	13.25–9.39	10
72.9–95.6	6.25–1.40	30	29.2–40.9	20.25–13.26	5
59.5–72.8	8.38–6.26	20	Below 29.2	More than 20.25	3
54.2–59.4	9.38–8.39	15			

[1] Precipitated with 0.5-percent H_2SO_4 in 95-percent ethanol.

[2] When the approximate concentration of rubber or resins in solution is not known, trial colorimeter readings at 4 minutes' standing time are made as a guide to the required final standing time indicated in the last column. In practice an extra aliquot is prepared for precipitation, and this is discarded after the trial reading is made.

[3] Precipitated with 0.5-percent H_2SO_4 in H_2O.

PROCEDURE FOR RESINS ANALYSIS

The procedure for resins analysis is the same as that indicated for rubber analysis except that the solvent is acetonylacetone-cellosolve 3–7; the precipitant is 0.5-percent H_2SO_4 in H_2O; and 0.1 ml. of the protective colloid solution for resins is added to each 25-ml. flask containing the aliquot of the extract before the precipitant is added.

PHOTOMETRIC INSTRUMENTS RECOMMENDED AND CALCULATION OF RESULTS

Photometric readings of percentage of light transmittance (T) are made on the Coleman double monochromator spectrophotometer at

750-mμ wave length; or readings are made on a colorimeter provided with the monochromatic filter combination for selecting the approximate wave length at 750 mμ. For routine determinations the Evelyn photoelectric colorimeter provided with a 720-mμ filter is recommended.

The liquid in which the precipitated rubber and resins are suspended is poured very carefully down the inside of the cuvette or absorption tube to avoid the formation of air bubbles. After rubber and resins precipitation, when the Evelyn photoelectric colorimeter is used, the readings are made after the standing times shown in table 2. It has been found convenient to precipitate rubber or resins from the aliquots at 2-minute intervals. This allows sufficient time to check the colorimeter and to make the readings. When a change is made from one reading to the next it is advisable to rinse the cuvette or absorption tube at least twice with small portions of the solution to be read.

After the colorimeter readings have been made the milligrams of rubber or resins or the percentages present in the aliquot are read from calibration curves (figs. 4 and 5) or from prepared tables of equivalents based in each case on experimental data. The results are expressed as percentages of dry weight (8 hours' drying at 100° C.) of the original tissue. The error due to leaving the insoluble residue in the volumetric extraction flask when the extract is being made up to volume is very slight since only 0.2 gm. of tissue is used. For routine analyses this very slight error may be disregarded.

EXPERIMENTAL RESULTS

The experimental results on which the methods of rubber and resins analysis are based are discussed briefly herein.

Preparation of Samples

No difficulty was experienced in preparing samples of nursery-grown guayule plants less than 2 years old for rubber and resins analysis. In the case of older field-grown guayule plants errors, however, may be introduced by the customary method of preparing the samples for analysis as is briefly indicated in this section. Such errors do not affect the precision of the methods for rubber and resins analysis, since they are due solely to the tissue-sampling procedure in the case of older field-grown plants. The method of sampling field-grown plants more than 2 years old, therefore, requires further investigation.

PARBOILING FOR REMOVAL OF LEAVES

Parboiling, which consists in boiling guayule plants in water for 8 to 10 minutes, is commonly used as a means of leaf removal prior to drying and grinding. It is, therefore, necessary to determine any possible effect of this procedure on the final results. Experiments have shown that there is a slight loss in dry weight of roots and stems. In a typical experiment branches of 3- to 4-year-old guayule plants were halved at random. One set of halves was parboiled and the leaves were hand-picked from the other set. Both sets were dried for 48 hours at 65° C. in a mechanical convection oven. The results are summarized as follows:

Plant:	*Dry weight lost in parboiling (percent)*
1	1.4
2	2.1
3	1.2
4	2.6
Mean	1.8

The effect of parboiling on the final analyses is summarized in figure 6. Rubber recovery was about 1 percent higher for parboiled plants and resins recovery somewhat over 1 percent lower. The difference in rubber recovery cannot be accounted for by loss in dry weight. A clue is to be found in a study of the grinding behavior of a parboiled and an unparboiled shrub. The grinding of the parboiled shrub in the hammer mill is much facilitated, and as a rule for tissue with a rubber content below 10 percent no aggregates of rubber develop in the machine during the operation. With the unparboiled shrub some difficulty may be experienced, since some of the sticky exudate on the plant may serve as nuclei for the formation of rubber aggregates. This apparently explains the apparent loss in rubber from the unparboiled shrub as compared with the parboiled (fig. 6). The loss in resins

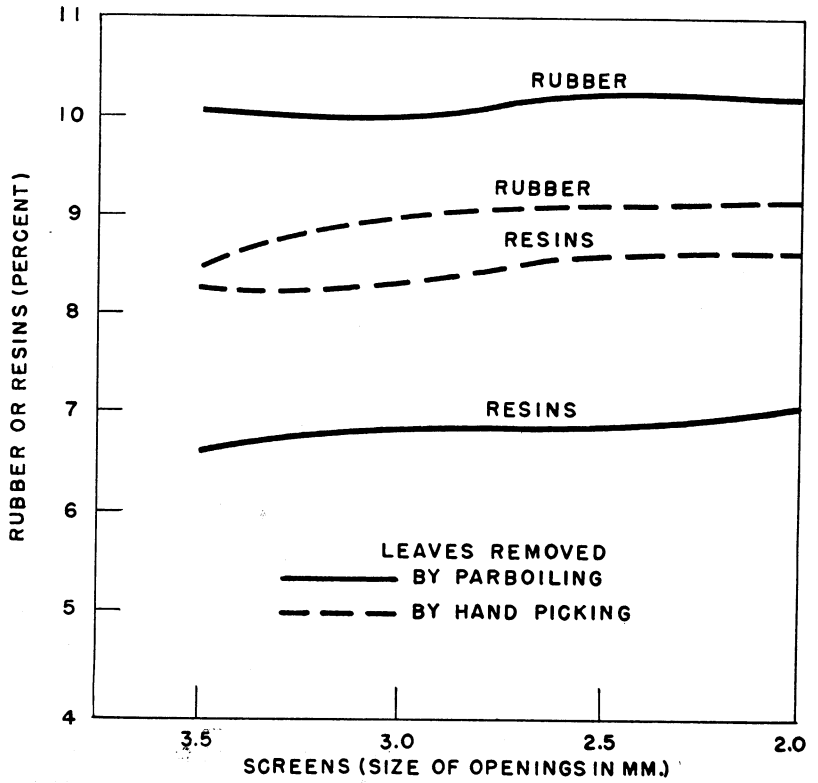

FIGURE 6.—Effect of particle size on recovery of rubber and resins when leaves were removed by parboiling and by hand picking (control); plants ground in a hammer mill.

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recovery from parboiled shrub may be due to the loss of the resinous exudate during parboiling. This exudate forms as a scum on top of the liquor in the tank.

On the basis of these results the recommendation is made not to vary the method of leaf removal in any one research project. Then fairly comparable results should be obtained.

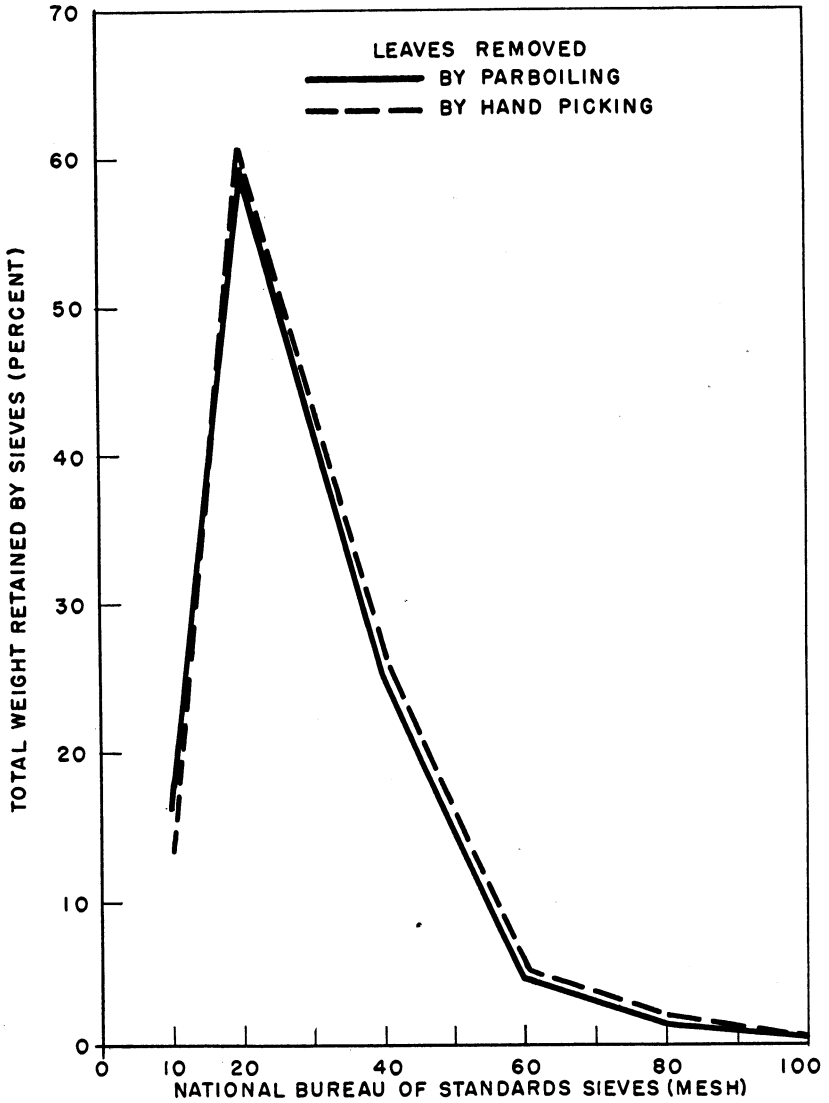

FIGURE 7.—Distribution of particle sizes of guayule tissue ground in a Ball & Jewel mill to pass six different screens; leaves removed from plants by parboiling and hand picking (control); defoliated plants dried to a moisture content of 0.5 to 0.9 percent; ground tissue shaken 10 minutes in a Fisher-Kahn shaker.

GRINDING

The entire plant or plant parts are given a preliminary drying for 24 hours at 65° C. prior to the preliminary grinding in a Wiley or a Ball & Jewell mill. An analysis of particle sizes after coarse grinding shows that they are about the same for parboiled and unparboiled shrubs (fig. 7).

The coarsely ground sample is then dried for 48 hours at 65° C., after which it has a moisture content of 2 percent or less as determined by drying subsamples at 100° for 8 hours. The sample is then ground in a hammer mill. The model used in this work is a Raymond mill as modified by Ross and Hardesty (*4*). Screens with round perforations are furnished by the Raymond Combustion Engineering Co. The sizes furnished include No. 1 (2 mm.), No. 2 (2.5 mm.), No. 3 (3.3 mm.), and No. 4 (4 mm.). An analysis of the results secured with these screen sizes with parboiled and unparboiled shrubs is shown in figure 8. This shows that screens Nos. 1 and 2 are similar but that

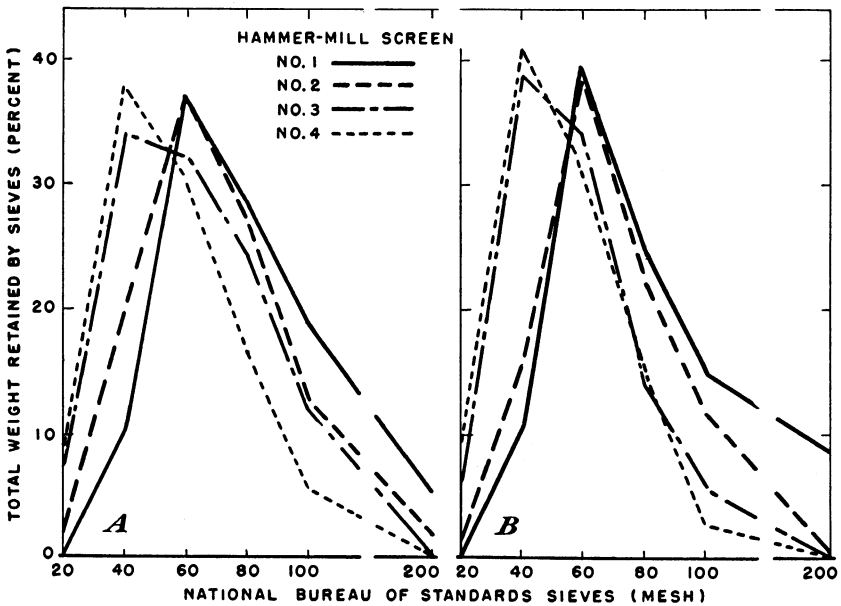

FIGURE 8.—Distribution of particle sizes of guayule tissue ground in a hammer mill to pass six different screens. Leaves removed by hand picking (control) (*A*) and parboiling (*B*); defoliated plants dried to 0.8 to 1.1 percent moisture content; ground tissues shaken 10 minutes in a Fisher-Kahn shaker.

No. 1 gives a definitely larger percentage of finer particles. Experiments have shown that for maximum extraction of rubber and resins from ground guayule tissue by the procedure outlined on page 14, 2- or 2.5-mm. screens should be used (fig. 6). The advantage of using No. 1 screen is that errors due to sampling the ground tissue are reduced as much as is possible with the present equipment.

It should be emphasized that in the case of samples with a rubber content of 10 percent or more it is not possible to use screen No. 1.

Even with screen No. 2 difficulty due to rubber aggregations may be encountered. In these cases it is best to parboil the shrub for leaf removal and finally to dry to about 1 to 2 percent of moisture for best results in grinding in the hammer mill.

It is advisable to provide the hammer mill with a ground wire, since the presence of static electric charges on the particles will cause some of these to adhere to the inside of the container or may cause some of the particles to form aggregates. Errors in sampling from this source may be considerable.

Solvents

Among the solvents with high boiling points that may be used in rubber analysis are methyl n-amyl ketone, methyl isobutyl ketone, diisobutyl ketone, butyl acetate, methyl amyl acetate, ethylene glycol diethyl ether, and n-butyl ether (table 1). These are also resins solvents, but the resins are held in solution by the rubber precipitant, ethanol, when rubber is precipitated (see tables 3 and 4). On the basis of the experiments the best all-round solvent for use in the present method is diisobutyl ketone, which was not manufactured during World War II. A suitable substitute is methyl isobutyl ketone, which is reported as nontoxic (*9*).

Cellosolve (ethylene glycol monoethyl ether), butyl "carbitol" (diethylene glycol monobutyl ether), and acetonylacetone may be used as resins solvents (table 1).

In the earlier work with resins analysis cellosolve alone was selected as a suitable solvent. Although cellosolve alone is excellent, its relatively greater viscosity leads to bubble formation after resins precipitation. This increased the experimental error. One of the other resins solvents, acetonylacetone, has the property of greatly reducing viscosity when added to other liquids. This solvent therefore was added to cellosolve in sufficient amount to overcome the difficulty. On the basis of experiments 3 parts of acetonylacetone to 7 parts of cellosolve (both by volume) was selected. The other solvent listed, butyl "carbitol," at least the technical-grade product used, is apparently not as good as either of the other two or a combination of these.

The solvents for use in analyzing artificial rubbers by the present method apparently depend on the kind of rubber. Mesityl oxide is suitable for Buna S and butyl ether for butyl rubber. These reagents are technical grade, Carbon & Carbide Chemicals Corp. This subject has not been extensively investigated by the writer.

Precipitants

Any test for the adequacy of the precipitants recommended must be based on the precision of the final results obtained with the methods. The precipitants selected after extensive experimentation are satisfactory (see fig. 16 and tables 6–9). Extensive tests carried out have shown that a satisfactory precipitant for rubber is 0.5-percent H_2SO_4 (c. p. reagent, Du Pont, sp. gr. 1.84) in 95-percent ethanol by volume. For guayule resins 0.5-percent H_2SO_4 in H_2O by volume is a satisfactory precipitant. A solution of 1 part of H_2SO_4 to 5 parts of H_2O (1–5 H_2SO_4) and also various intermediate proportions of these substances give fair results, but with the increase in the ratio of H_2SO_4

to H_2O the solution becomes somewhat viscous and precautions must be taken to avoid bubble formation when it is poured into the cuvettes.

It should be noted that both of these precipitants have an acid reaction. It was found that when NH_4OH was used in the rubber precipitant in place of H_2SO_4 the readings were stable but were not reproducible. The use of NaOH in place of H_2SO_4 gave variable results as a rule. However, when the 95-percent ethanol in rubber analysis or H_2O in resins analysis was acidified the characteristics of the liquid with precipitated rubber or resins in suspension as read on the spectrophotometer or colorimeter followed a regular course as explained on page 24 (see figs. 11 and 12). This made it possible to obtain reproducible results and consequently the required degree of precision.

PROTECTIVE COLLOIDS

In the earlier experiments, before the solvents and precipitants had been properly adjusted, it was found that the use of a protective colloid such as diglycol stearate S improved the reproducibility of the results markedly. Out of a great many similar compounds tested diglycol stearate S apparently proved to be the most useful. However, after the final procedures were developed, the benefit of the protective colloid was not so greatly needed. In the case of resins determinations it is advisable to use it, and a slight benefit may be secured by its use in rubber analysis. The details for the preparation of the protective colloid solutions and concentrations recommended are given on page 5.

Eliminating Errors Due to Extraneous Colors

INTERFERING COLORS

One of the main problems that had to be solved in working out the methods for rubber and resins analysis in plant samples was to eliminate the error due to the presence of extraneous colors. The amount of color varies from sample to sample depending on the age of the material, the part of the plant sampled, the history of the plant material subsequent to harvesting, and possibly other factors.

In rubber analysis this error can be overcome by first extracting the sample with acetone or cellosolve, which removes the coloring matter and the resins. This, however, requires an extra step in procedure. In resins analysis apparently there is no easy procedure for the removal of chlorophyll and other coloring matters that may be present without also affecting the resins content.

The problem was first solved by selecting the proper wave length on the Coleman double monochromator spectrophotometer for the determination of the percentage of light transmittance (T) after precipitation of rubber and resins from solutions containing various amounts of coloring matter. In place of the spectrophotometer a colorimeter may be used in routine analytical procedure if it is provided with the necessary monochromatic filter combination.

SELECTION OF WAVE LENGTHS

Spectral transmittance curves for rubber extracts in methyl isobutyl ketone and for resins extracts in acetonylacetone-cellosolve 3–7 from various types of guayule tissue are shown in figure 9. The results for cellosolve alone are similar to those for the mixture indicated.

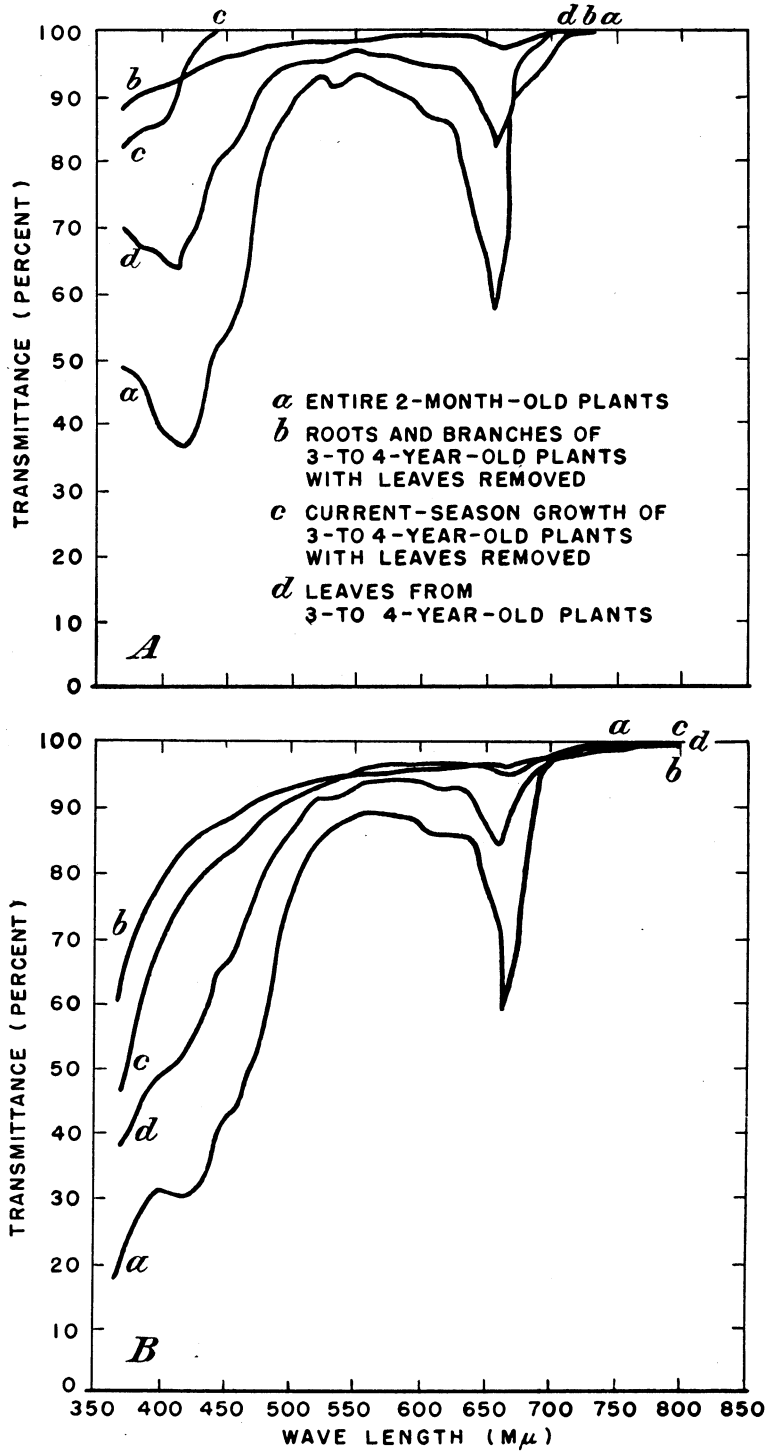

FIGURE 9.—Spectral transmittance curves for extracts from 0.2 gm. of guayule tissue in a 25-ml. volume of (*A*) methyl isobutyl ketone and (*B*) acetonylacetone-cellosolve 3–7.

The data presented indicate that for the various methyl isobutyl ketone extracts the regions of maximum absorption when all tissues are considered are between wave lengths 350 to 475 mμ and 625 to 675 mμ, whereas the regions of greatest light transmittance are between wave lengths 475 to 625 and 700 mμ and above. Similar results were secured for acetonylacetone-cellosolve 3–7 extracts of guayule tissue. The error due to color in the liquid after rubber or resins have been precipitated may be reduced to the vanishing point by selecting, for instance, wave bands above 700 mμ. Actual experiments have shown that the most suitable wave band for use in rubber and resins analysis is in the neighborhood of 750 mμ.

A summary of a typical experiment to show effective control of errors due to color in rubber and resins extracts from guayule tissue is presented in table 3. The solvents, acetone and cellosolve, which are reported as nonrubber solvents, extract the colored materials and resins. This makes it possible after the evaporation of the solvents to take up residues of such extracts in the rubber solvents and thus secure a solution with the color but without any rubber. When the rubber precipitant is then added one has a direct test of the efficiency of wave length 750 mμ in eliminating errors due to extraneous color.

TABLE 3.—*Effect of adding acetone-extract residue of tissue of 3- to 4-year-old guayule plants to methyl isobutyl ketone or of cellosolve extract of such tissue on percentage of light transmittance (T) as determined on a Coleman double monochromator spectrophotometer at 750-mμ wave length and 5-mμ band width*

Ingredients made up to 25-ml. volume		Light transmittance (*T*) when sample is from indicated plant part		
Name	Amount	Root, stem, and branches	New growth	Leaves
	Ml.	*Percent*	*Percent*	*Percent*
Acetone-extract residue without rubber: [1]				
Acetone-extract residue in methyl isobutyl ketone [2]	5			
Methyl isobutyl ketone	5	100	100	100
Diglycol stearate S solution	.1			
Acidified ethanol (precipitant)	14.9			
Rubber without acetone-extract residue: [1]				
Methyl isobutyl ketone	5			
2 mg. of rubber in methyl isobutyl ketone	5	62.6	62.8	62.0
Diglycol stearate S solution	.1			
Acidified ethanol (precipitant)	14.9			
Acetone-extract residue plus rubber: [1]				
Acetone extract residue in methyl isobutyl ketone [2]	5			
2 mg. of rubber in methyl isobutyl ketone	5	61.9	61.9	61.9
Diglycol stearate S solution	.1			
Acidified ethanol (precipitant)	14.9			
Cellosolve extract without rubber: [3]				
Cellosolve extract [4]	5			
Methyl isobutyl ketone	5	100	100	100
Diglycol stearate S solution	.1			
Acidified H_2O (precipitant)	14.9			
Rubber without cellosolve extract: [3]				
Cellosolve	5			
2 mg. of rubber in methyl isobutyl ketone	5	62.3	62.8	62.8
Diglycol stearate S solution	.1			
Acidified H_2O (precipitant)	14.9			
Cellosolve extract plus rubber: [3]				
Cellosolve extract [4]	5			
2 mg. of rubber in methyl isobutyl ketone	5	62.0	62.0	62.4
Diglycol stearate S solution	.1			
Acidified H_2O (precipitant)	14.9			

[1] Blank: 10 ml., methyl isobutyl ketone; 0.1 ml., diglycol stearate S solution; and 14.9 ml., acidified ethanol
[2] 0.2 gm. of guayule tissue extracted with 40 ml. of acetone; acetone evaporated off and residue taken up in methyl isobutyl ketone and made up to 50-ml. volume with methyl isobutyl ketone.
[3] Blank: 5 ml., cellosolve; 5 ml., methyl isobutyl ketone; 0.1 ml., diglycol stearate S solution; and 14.9 ml., acidified H_2O.
[4] 0.2 gm. guayule tissue extracted with 40 ml. cellosolve and made up to 50-ml. volume with cellosolve.

In table 3 is presented such direct evidence showing that the color extracted from guayule tissue with acetone and cellosolve is effectively screened out by the selection of wave length 750 mμ on the spectrophotometer. This table also shows that the rubber added can be quantitatively recovered in the presence of the colored materials and resins and that rubber was apparently not extracted from the guayule tissue by these solvents. These results give convincing evidence that the use of wave length 750 mμ on the spectrophotometer is the proper procedure in photometric analysis of rubber or resins.

SELECTION OF MONOCHROMATIC FILTER COMBINATION

On the basis of the results shown in table 3 attempts were made to secure the proper monochromatic filter combinations for various colorimeters. The 720-mμ filter furnished for the Evelyn photoelectric colorimeter, for which the characteristics of the relative light transmission (t) curve are shown in figure 10, gives satisfactory results. The 750-mμ filter combination, for which the relative light transmission curve is also shown in figure 10, is satisfactory; but this is not standard equipment.

Up to the present useful filter combinations in the 700-mμ range have not been secured for the Klett-Summerson colorimeter, but further attempts are being made.

TIME OF STANDING AFTER RUBBER OR RESINS PRECIPITATION

A consideration of the time of standing after precipitation of rubber or resins from solutions is important, for the percentage of light trans-

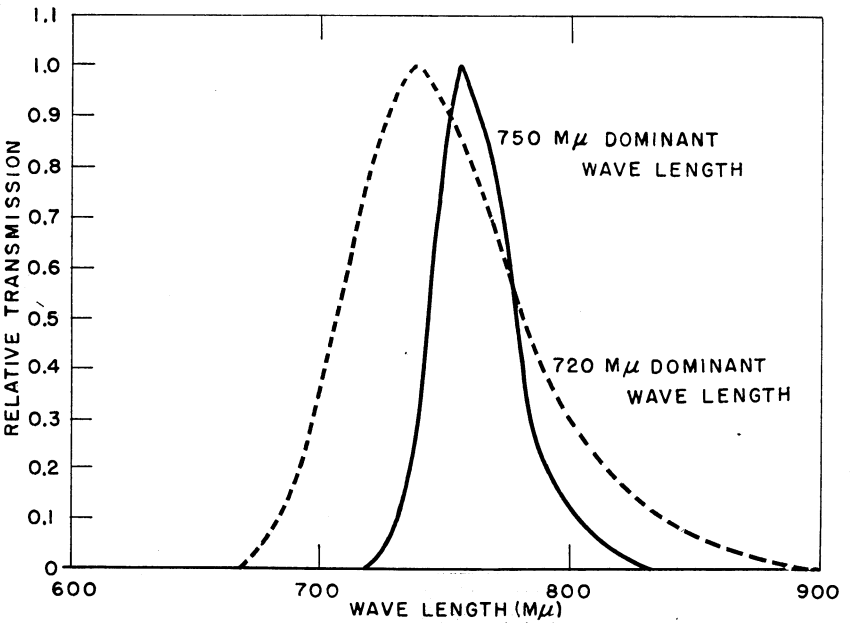

FIGURE 10.—Comparative spectral transmission (t) curves for filter combinations with dominant wave lengths at 720 and 750 mμ.

mitted (spectrophotometer readings) or light scattered (photofluorometer readings) is subject to change.

Figures 11 and 12 show that in the case of transmitted light under the experimental conditions there is first a decrease in percentage of transmittance followed by a period of relative stability with only slight changes after a number of minutes. The light transmittance then increases. This may be summarized as follows: *The percentage of light transmitted through acidified liquids with precipitated rubber or resins in suspension is not stable; the time required after precipitation of rubber or resins to reach a comparatively stable period for transmitted light and the duration of this period vary inversely with the concentration of the rubber or resins suspended in the liquids.* For scattered light on the photofluorometer the direction of change is the reverse of that indicated for transmitted light. It can be readily seen that if readings are made after the solutions reach this period of relative stability the error from this source can be reduced to the minimum. The reading times based on these data for rubbers and resins are given in table 2.

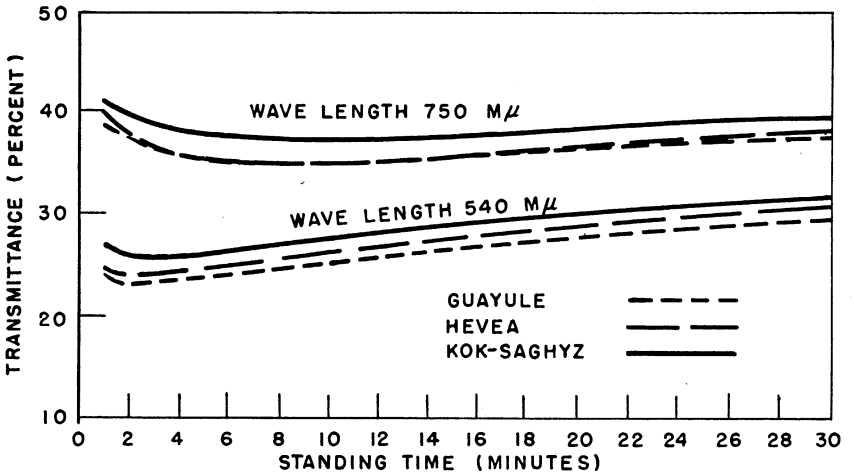

FIGURE 11.—Effect of wave length on light transmittance after different standing times for guayule, hevea, and kok-saghyz rubbers precipitated from methyl isobutyl ketone solutions by acidified ethanol (4 mg. per 25-ml. volume). (Readings on Coleman double monochromater spectrophotometer with 5-mμ wave band.)

NIL EFFECT OF RESINS IN SOLUTION

The rubber solvents extract the resins also. It has already been stated that the resins are soluble in ethanol and therefore are not precipitated with the rubber (table 3). There is still the question of any possible effect of extreme differences in concentrations of resins in solution along with the rubber. The data in table 4 show, on the basis of colorimeter readings, that the amount of rubber precipitated from methyl isobutyl ketone solution by 0.5-percent H_2SO_4 in 95-percent ethanol is not affected by the presence of varying amounts of resins in solution.

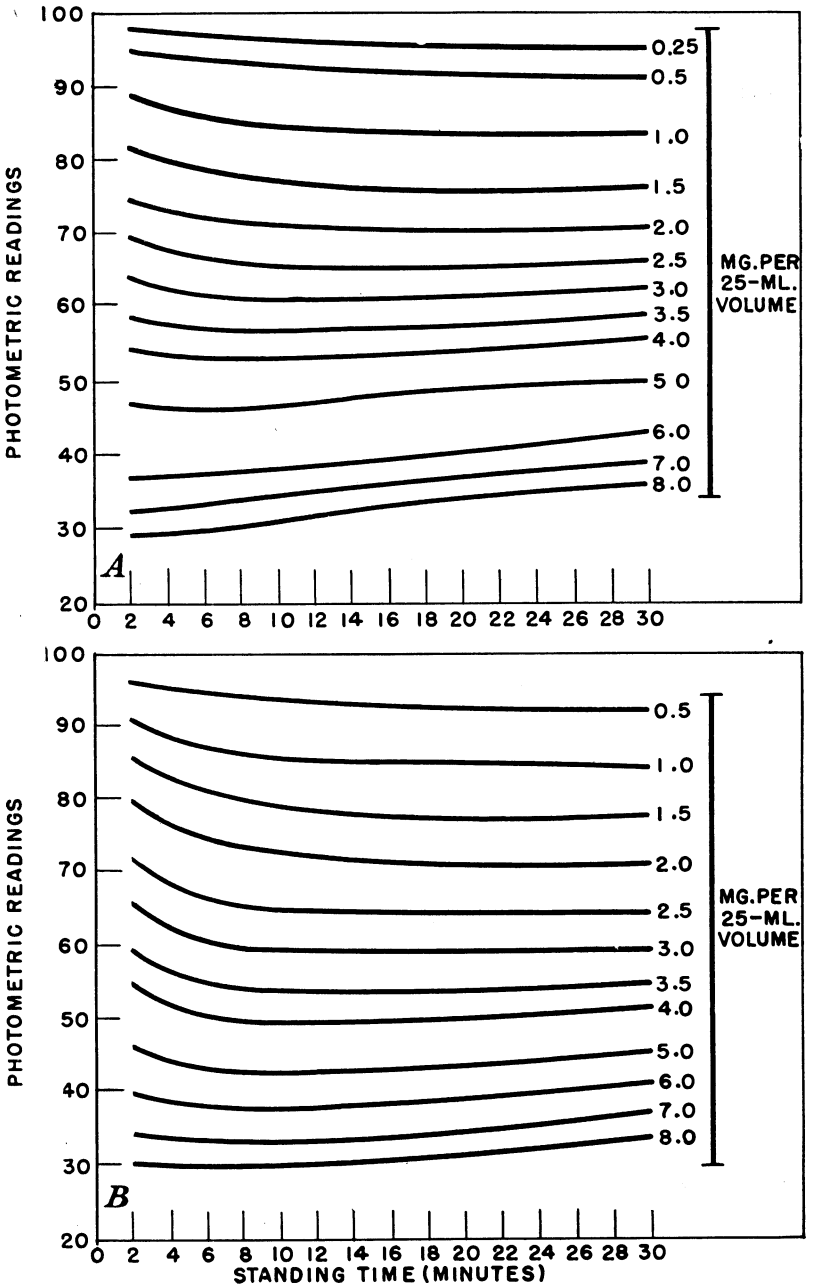

FIGURE 12.—Effect of time of standing on light transmittance for (*A*) guayule rubber precipitated from methyl isobutyl ketone solution by acidified ethanol and (*B*) guayule resins precipitated from acetonylacetone-cellosolve 3–7 solution by acidified H_2O. (Readings at 2-minute intervals on Evelyn photoelectric colorimeter with 720-mμ filter and 6-ml. aperture; see also table 2.)

TABLE 4.—*Relation of amount of rubber precipitated from methyl isobutyl ketone solutions by 0.5-percent H_2SO_4 in 95-percent ethanol to amount of resins added, as indicated by colorimetric readings*

[Rubber and resins calculated on basis of amounts in 5-ml. aliquots of extract from 0.2 gm. of tissue in a 25-ml. volume]

Rubber added (percent)	Rubber recovered when resins added as indicated				
	0	2.5 percent	5.0 percent	7.5 percent	10.0 percent
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
0	0	0	0	0	0
2.50	2.50	2.50	2.53	2.52	2.50
5.00	5.00	5.00	5.00	5.00	5.00
7.50	7.50	7.50	7.50	7.51	7.49
12.50	12.50	12.50	12.50	12.50	12.50

EXTRACTION TEMPERATURE AND TIME

Typical experiments to determine the time required to secure maximum extraction of rubber and resins at 115° C. are summarized in figure 13; the solvents used—methyl isobutyl ketone for rubber and acetonylacetone-cellosolve 3–7 for resins extraction—are very efficient since under the conditions indicated maximum values were secured after 30 minutes of extraction. The most efficient liquid for the bath (fig. 1) used in extracting rubber or resins is a high-grade cylinder oil (S. A. E. 20). The sand bath and such liquids as glycerol have proved unsatisfactory.

RUBBER AND RESINS STANDARDS

According to the National Bureau of Standards [14]—

> There is no generally recognized standard material [for a rubber standard]. The rubber hydrocarbon in different samples of commercial smoked sheet or pale crepe from hevea plantations varies from 92 to 94 percent of the total under the most favorable conditions of preparation.

McPherson (*2*) has described a procedure for the preparation of pure hevea (*Hevea brasiliensis* (H. B. K.) Muell. Arg.) rubber from crude rubber or latex. In the absence, however, of a generally recognized standard for rubber hydrocarbon [caoutchouc, $(C_5HU_8)_x$] the standard adopted was that of the relatively pure product secured from guayule tissue extraction according to the Spence-Caldwell method (*5*) or the modified procedure of Holmes and Haasis (p. 2). The details for constructing the calibration curves are given on page 10. Standard rubber samples apparently can be stored for a long time under the conditions described on page 10. The duplicate colorimetric readings for guayule rubber newly extracted by the modified Spence-Caldwell method were 69.2 each as compared with 68.9 each for the sample extracted by the same method but stored 1¾ years. In each case colorimeter readings were made on mixtures containing 2 mg. of rubber in a 25-ml. volume.

The selection of a suitable standard for guayule resins presents some difficulties. The acetone extract of guayule, estimated on a gravimetric basis, for instance, proved to be variable, depending on the type of tissue sampled or season of sampling, because such fractions as

[14] Communication dated October 22, 1943.

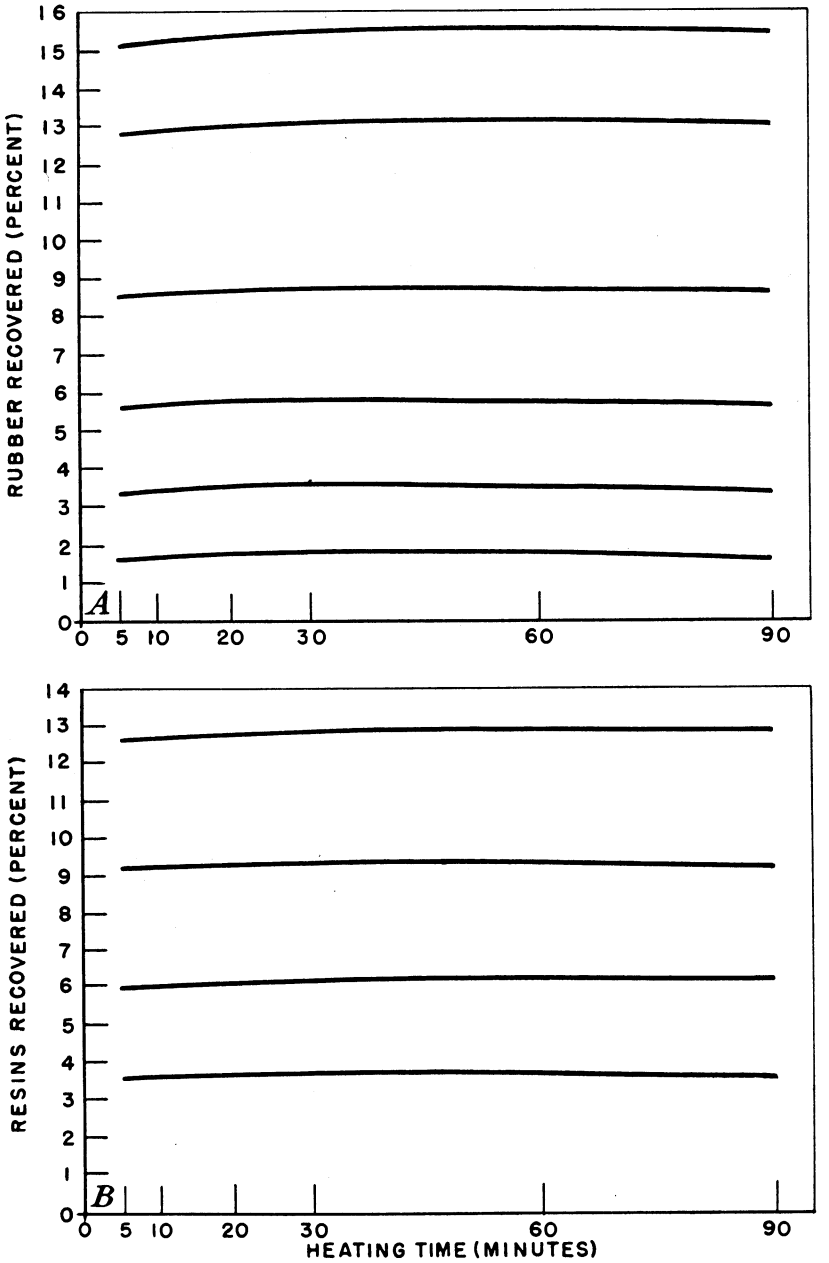

FIGURE 13.—Effect of time of heating 0.2-gm. samples of guayule tissue with solvent at 115° C. on recovery of rubber and resins: *A*, Rubber extracted with methyl isobutyl ketone; *B*, resins extracted with acetonylacetone-cellosolve 3–7.

chlorophyll are weighed along with the resins in the acetone extract but are not estimated by the present photometric method (fig. 9). The use of a different standard was therefore indicated.

The resins standard selected is based on a relatively pure source of resins, the resinous exudate on the stems of guayule plants. Preliminary experiments with the resinous exudate from different guayule strains of various ages indicate that there is little variation in this substance on the basis of colorimeter readings on 2-mg. aliquots of 0.1-percent solution in a 25-ml. volume (table 5). Experiments have shown that the air-dried exudate contains 3.8 ± 0.14 percent of volatile matter. This material is relatively stable in composition and is apparently only slightly hygroscopic as shown in table 5. The resinous exudate may be prepared in two ways for use as a standard—(1) the clean exudate is dried for 6 hours at 65° C. at 30 inches of vacuum and after being ground in a mortar is preserved in an airtight container or (2) the exudate is dissolved in acetone and filtered. Practically all of the acetone is then evaporated off over a steam or water bath, and the residue is dried, ground, and preserved as in the first method. These two preparations are compared in table 5. The details for storing the resins standard and constructing the calibration curves are given on page 12.

TABLE 5.—*Comparison of air-dried and vacuum-oven-dried resins exudates of guayule*

[Colorimetric readings on Evelyn photoelectric colorimeter with 720-mμ (690- to 770-mμ) filter and 5-ml. aperture; precipitant 1 part of 0.5-percent H_2SO_4 to 5 parts of H_2O]

Strain No.	Age of plants	Treatment of resins exudate	Duplicate colorimeter readings (based on 2 mg. of resins exudate per 25-ml. volume) [1]
	Years		
Various	3–9	Composited; air-dried	66.2; 66.2
Do	3–9	Composited; dried at 65° C. in 30-inch vacuum for 6 hours.[2]	66.5; 66.3
Do	3–9	Composited; dissolved in acetone; filtered; acetone evaporated off over steam bath; residue dried at 65° C. in 30-inch vacuum for 6 hours.	66.0; 66.0
406	1–2	Air-dried	66.9; 66.7
593	1–2	do	66.4; 66.4
406	3–4	do	65.8; 65.8
593	3–4	do	66.0; 66.0
406	8–9	do	66.2; 66.0
593	13–14	do	66.3; 66.3
406	13–14	do	65.8; 66.0

[1] The values in the last column indicate that guayule resinous exudate, whether dried in vacuum with 3.8 percent loss of volatile matter or not dried, gives approximately the same colorimeter readings on the basis of aliquots containing 2 mg. of resins exudate per 25-ml. volume. This indicates that the same mass of volatile matter precipitated gives approximately the same colorimeter readings as the residue. A preliminary experiment indicates that the volatile matter consists of the "terpene oil" fraction: 15-gm. samples of resinous exudate gave by steam distillation an average of 0.7 ml. of "terpene oil" with approximate density of 0.86 and thus represented approximately 4 percent of the original weight. This percentage compares with 3.8 percent lost by drying in vacuum.

[2] 3.8 ± 0.14 percent of original weight lost.

The available information on the constituents of the guayule resinous exudate and the acetone extract of guayule tissue is summarized in this paragraph. Walter (*6*) and also Haagen-Smit and his coworkers [15] reported that 20 percent of the resinous exudate from guayule stems is parthenyl cinnamate ($C_6H_5CH=CHCO_2C_{15}H_{25}$). Steam dis-

[15] Communication from A. J. Haagen-Smit and coworkers, California Institute of Technology, Pasadena. These workers are studying the constituents of the acetone extract and other constituents of guayule.

tillation of similar resinous exudate in the writer's laboratory has yielded approximately 4 percent of the whole as the "terpene oil" fraction. Haagen-Smit and his coworkers reported that about 2.5 percent of the acetone extract from guayule branches is parthenyl cinnamate and 6 percent of the bark (cortex-phloem) extract is parthenyl cinnamate. These workers further reported that 2.5 percent of the acetone extract from branches is a compound with the empirical formula $C_{30}H_{46}O_3$; 1 percent is a compound melting at 271° to 275° C.; and 1.5 percent of the extract from the leaves is a compound melting at 170° to 171°. Walter (*6*) reported that 3 to 4 percent of the acetone extract of leaves and flowering stems of guayule consists of waxlike substances soluble in organic solvents but insoluble in water and concentrated H_2SO_4. The fractions mentioned, on the basis of conservative estimates of amounts actually isolated, accounted for about 8 percent of the total acetone solubles extracted from the guayule shrub. The other constituents remain to be identified.

Rubber and Resins Calibration Curves

Typical calibration curves for rubber and resins based on determinations made according to the procedure outlined on pages 4 to 16 are shown in figures 5 and 14. The spectrophotometer or colorimeter readings are plotted on semilogarithmic paper against the rubber con-

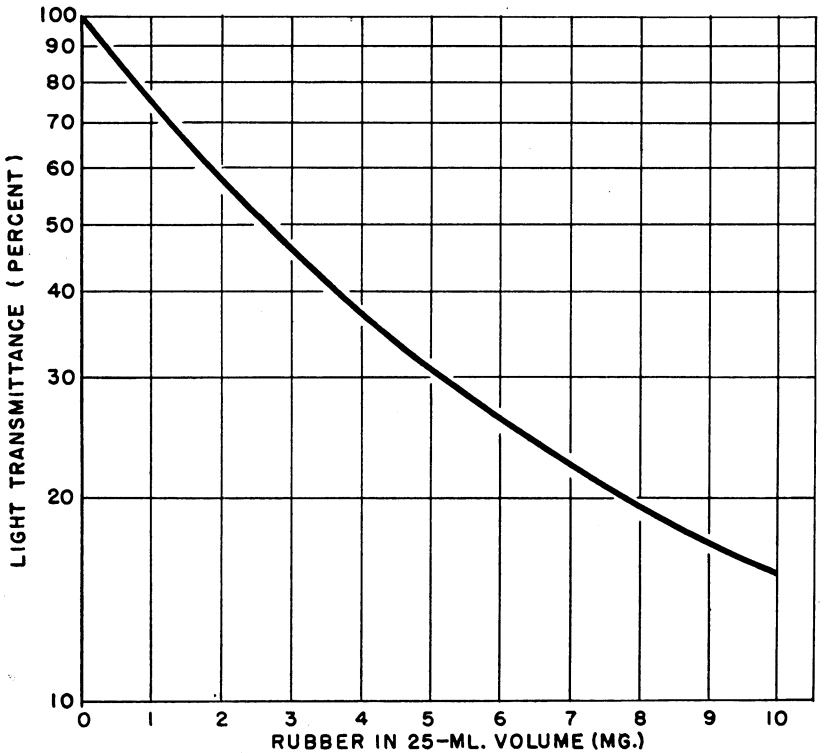

FIGURE 14.—Calibration curve for guayule rubber. (Readings on Coleman double monochromator spectrophotometer with 750-mμ filter and 5-mμ wave band.)

centrations. From a casual inspection of these curves it would appear that Beer's law does not hold. Actual tests indicated that Beer's law was applicable only within a narrow range in the upper portion of the curves. The analytical results on the basis of photometric readings are therefore calculated by direct reference to calibration curves or from reference tables of equivalent values based on the calibration curves.

Calibration curves for kok-saghyz and hevea natural rubbers and Buna S (GR–S; Chem. Gum IV, Goodyear) and butyl (GR–I, Standard Oil Co., Protoc) synthetic rubbers are shown in figure 15. The samples of kok-saghyz rubber were furnished by the Division of Rub-

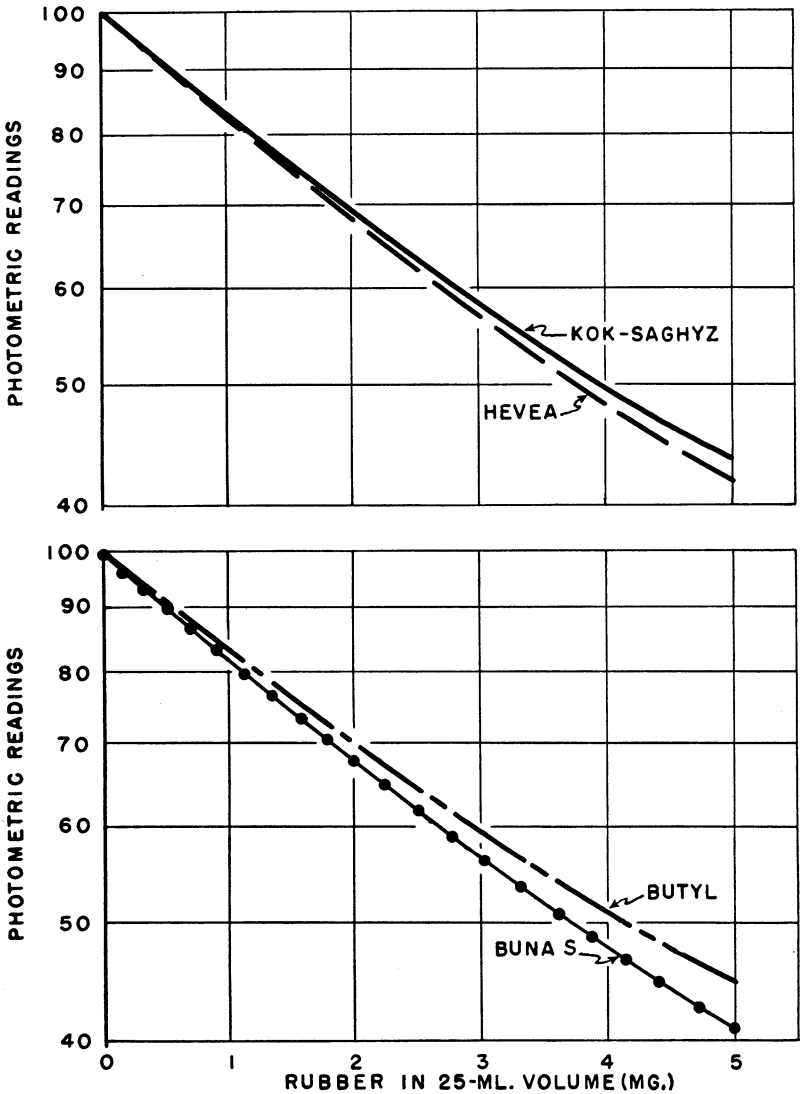

FIGURE 15.—Calibration curves for kok-saghyz, hevea, Buna S, and butyl rubbers. (Readings on Evelyn photoelectric colorimeter with 720-mμ filter and 6-ml. aperture.)

ber Plant Investigations of this Bureau and were prepared according to the Spence-Caldwell (*5*) procedure. Hevea pale crepe was extracted with acetone before it was used as a standard.

Sensitivity of Methods

By the procedure outlined on page 4 as low as 0.001 mg., or 1 microgram (gamma), of rubber in a 25-ml. volume can be detected on the Coleman double monochromator spectrophotometer; similarly, 0.01 mg. of rubber in a 25-ml. volume may be read on the Evelyn photoelectric colorimeter. These values, on the basis of extraction from 0.2 gm. of tissue, according to the standard procedure, represent 0.0025 and 0.025 percent of rubber, respectively. Although such low values can be detected, it should be realized that the error in percentage of the amount determined is relatively high in this very low range. In actual practice the lower limit, under the conditions of the procedure as outlined, is set at 0.04 mg. of rubber in a 25-ml. volume, representing 0.1 percent of rubber on the basis of extraction from 0.2 gm. of tissue.

Error Considerations

In order to secure the greatest precision in results, it is necessary to standardize rigidly the procedure as indicated on pages 4 to 16. If this is done, the experimental error is reduced to a minimum.

The precision of the present method on the basis of pure rubber and resins solutions can be judged from figure 16, which shows the average deviation of a single measurement in percentage of the amount of rubber or resins determined. The error percentages are given for 0.04 to

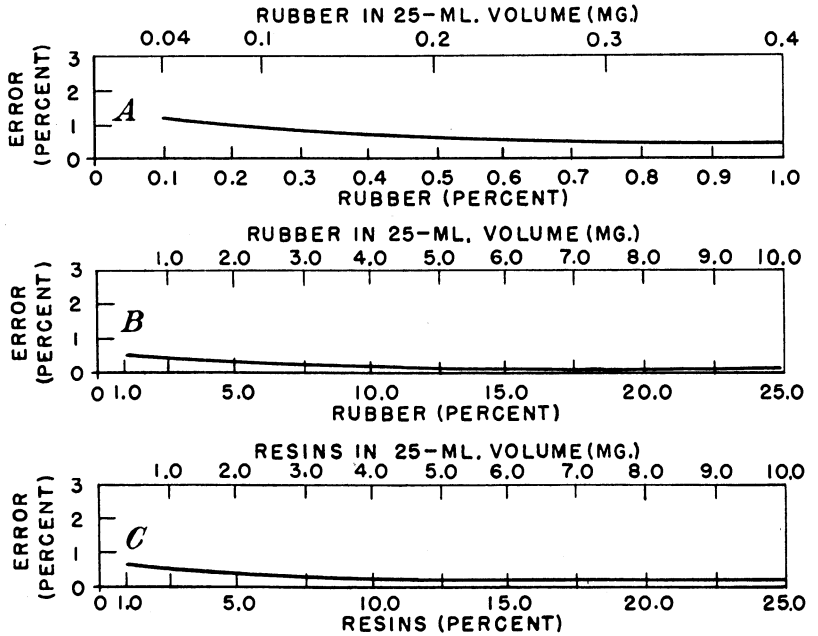

FIGURE 16.—Average deviation of a single measurement of pure rubber or resins solution on basis of 0.2 gm. of tissue (percent error) for (*A*) 0.04 to 0.4 mg. of rubber, (*B*) 1 to 10 mg. of rubber, and (*C*) 1 to 10 mg. of resins. (Readings on Evelyn photoelectric colorimeter with 720-mμ filter and 6-ml. aperture.)

10 mg. of rubber and 1 to 10 mg. of resins in a 25-ml. volume. For comparative purposes the results are also calculated on the basis of percentage rubber or resins in 0.2 gm. of tissue. As would be expected, the error increases somewhat for relatively low concentrations of rubber or resins. The curves in figure 16 show that in the range 0.1 to 1.0 percent of rubber the error ranges from about 1.2 to 0.5 percent; above this range the error decreases to considerably less than 1 percent of the amount determined. Similarly, in the case of resins analysis (fig. 16) the error is considerably less than 1 percent in the range of 1.0 to 25.0 percent.

In the case of rubber and resins extracted from tissues or added to tissue extracts (see tables 6–9), involving more operations, the error is higher than that indicated in figure 16 for pure rubber and resins solutions.

Correction for Insoluble Residue

In the procedure outlined on page 4 the insoluble tissue residue after extraction is left in the flask when the extract is made up to volume. It is not convenient to recover quantitatively the ketone and cellosolve extracts of guayule tissue and then make them up to volume, because such an additional step would be time-consuming. The logical alternative is to make the extract and residue up to volume in the original flask and if necessary to make a correction, when calculating the results, for the volume displaced by the insoluble residue. In a typical experiment, consisting of five replications, it was found that at 25° C. 0.2 gm. of rubber plus resin-free tissue (corrected for moisture on basis of drying at 100° for 8 hours) displaced only a mean volume of 0.08792 ± 0.00066 ml. of methyl isobutyl ketone (technical-grade; sp. gr. 0.7951 ± 0.0016 at 25°). Since the amount of tissue extracted is small, the error due to the insoluble residue is small. In routine work it can be disregarded.

Recovery of Rubber and Resins Added to Extract of Guayule Tissue

Table 6, representing typical results, shows that an average of 100.8 percent of the pure rubber added to the methyl isobutyl ketone extract of guayule tissue was recovered. This variation from complete recovery is within the experimental error. Similarly, table 6 shows that an average of 100.2 percent of guayule resins added to the cellosolve extract of guayule tissue was recovered.

Table 6.—*Recovery of rubber or resins added to samples of extract from 0.2 gm. of guayule tissue*

Instrument	Initial rubber content	Rubber added	Rubber determined	Added rubber recovered		Initial resin content	Resins added	Resins determined	Added resins recovered	
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Pct.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Pct.*
Coleman monochromator spectrophotometer with 750-mμ wave-length setting and 5-mμ wave band	20.0	10.0	30.1	10.1	101.0	14.85	10.0	24.90	10.05	100.5
	20.0	10.0	30.2	10.2	102.0	14.85	10.0	24.90	10.05	100.5
	21.0	10.0	30.8	9.8	98.0	15.2	10.0	25.20	10.00	100.0
	21.0	10.0	31.0	10.0	100.0	15.2	10.0	25.25	10.05	100.5
Evelyn photoelectric colorimeter with 720-mμ filter	19.75	10.0	29.9	10.15	101.5	7.16	5.0	12.25	5.09	101.8
	19.75	10.0	30.2	10.45	104.5	7.16	5.0	12.13	4.97	99.4
	19.0	10.0	29.0	10.0	100.0	13.94	10.0	23.87	9.93	99.3
	19.0	10.0	28.9	9.9	99.0	13.94	10.0	23.90	9.96	99.6

Comparison of Photometric and Modified Spence-Caldwell Methods of Rubber Analysis

Guayule samples were analyzed by the photometric method and by the modified Spence-Caldwell method. The values in table 7, representative of typical results, indicate a satisfactory degree of conformity between the two methods in the range of tissues with more than 0.9 percent of rubber. On the basis of the amounts determined, the results in this range for the present method average approximately 5 percent higher.

Table 7.—*Comparison of photometric and modified Spence-Caldwell methods of determining rubber content of guayule*

Sample class and No.	Rubber according to—		Difference (based on photometric method)	Sample class and No.	Rubber according to—		Difference (based on photometric method)
	Photometric method	Modified Spence-Caldwell method			Photometric method	Modified Spence-Caldwell method	
Samples with less than 1 percent of rubber:	*Percent*	*Percent*	*Percent*	Samples with more than 1 percent of rubber:	*Percent*	*Percent*	*Percent*
5222 [1]	0.12	0	−100	7982-A	2.74	2.68	−2
	.12	.11	−8		2.74	2.71	−5
R-5902	.17	.11	−35	R-8338	5.40	5.17	−4
	.17	.14	−18		5.42	5.28	−3
5221	.37	.19	−49	R-5482-A	6.47	6.09	−6
	.37	.22	−41		6.34	6.08	−4
R-17633	.40	.42	+5	R-5481-A	7.80	7.29	−7
	.42	.49	+17		7.86	7.36	−6
R-5905	.62	.50	−19	4744-1	8.87	8.50	−4
	.63	.65	+3		8.94	8.56	−4
R-5895	.89	.79	−11				
	.94	.90	−4				

[1] Flowers from 3-month-old nursery plants.

Variation of Single Determinations From Their Mean

Rubber analysis.—The precision of the present method when guayule tissues are analyzed for rubber may be judged from the data presented in table 8. These data show that the percentage variation of single determinations from mean values with low standard errors ranges from less than 1 to 4 percent.

Resins analysis.—As indicated on page 27, the results secured by the present photometric resins method are not strictly comparable with those of the gravimetric, acetone-extraction method. This made it necessary to select another calibration standard—the resinous exudate from guayule shrub—that gives relatively constant photometric readings (table 5). The resins contents of guayule roots, branches, or leaves usually range from about 3 to 14 percent. The precision of the present method when guayule tissues are analyzed for resins may be judged from the data presented in table 9. These data show that the percentage variation of single determinations from mean values with low standard errors ranges from about 0.5 to 4 percent.

TABLE 8.—*Variation in replicate rubber analyses of guayule tissue by the photometric method*

Sample No.	Rubber [1]	Variation from mean	Sample No.	Rubber [1]	Variation from mean
	Percent	*Percent*		*Percent*	*Percent*
1130–a	0.14	+3.7	1107–a	5.00	−2.2
1130–b	.13	−3.7	1107–b	5.25	+2.7
1130–c	.13	−3.7	1107–c	5.20	+1.8
1130–d	.14	+3.7	1107–d	5.00	−2.2
Mean	[2] .135±0.0029		Mean	5.11±0.066	
1135–a	.59	−3.8	1112–a	10.50	−2.4
1135–b	.63	+2.8	1112–b	10.75	−0.1
1135–c	.63	+2.8	1112–c	10.90	+1.3
1135–d	.60	−2.1	1112–d	10.90	+1.3
Mean	.613± .010		Mean	10.76± .094	
1155–a	.98	−0.3	1117–a	15.75	−1.3
1155–b	.98	−0.3	1117–b	16.30	+2.2
1155–c	.98	−0.3	1117–c	16.00	+0.3
1155–d	.99	+0.7	1117–d	15.75	−1.3
Mean	.983± .003		Mean	15.95± .131	
1102–a	2.50	−3.8	1122–a	21.60	+2.1
1102–b	2.70	+3.8	1122–b	20.80	−1.7
1102–c	2.50	−3.8	1122–c	21.60	+2.1
1102–d	2.70	+3.8	1122–d	20.60	−2.6
Mean	2.60± .058		Mean	21.15± .263	

[1] These values are for samples separately extracted according to the procedure outlined on pages 4 to 16.
[2] Second number represents standard error of the mean.

TABLE 9.—*Variation in replicate resins analyses of guayule tissue by the photometric method*

Sample No.	Resins [1]	Variation from mean
	Percent	*Percent*
8458–a	3.55	+4.1
8458–b	3.30	−3.2
8458–c	3.50	+2.6
8458–d	3.30	−3.2
Mean	[2] 3.41±0.066	
S–942–a	8.80	−0.6
S–942–b	8.85	0
S–942–c	8.75	−.6
S–942–d	9.00	+1.7
Mean	8.85± .054	
15980–a	9.75	+.6
15980–b	9.75	+.6
15980–c	9.50	−2.0
15980–d	9.75	+.6
Mean	9.69± .063	
R–17635–a	10.50	−.1
R–17635–b	10.50	−.1
R–17635–c	10.55	+.4
R–17635–d	10.50	−.1
Mean	10.51± .013	
6234–a	14.20	+.4
6234–b	14.10	−.4
6234–c	14.10	−.4
6234–d	14.20	+.4
Mean	14.15± .029	

[1] These values are for separately extracted samples according to procedure outlined on pages 4 to 16.
[2] Second number represents standard error of the mean.

Rubber Analysis in Crude-Rubber Products

New rapid photometric methods for the determination of rubber and resins in guayule tissue have been presented on pages 4 to 16. A brief summary of a similar rapid method for rubber analysis of unvulcanized crude rubber products, which is based also on the principles detailed there, follows: Duplicate 0.1-gm. samples of the finely divided material are weighed out. (Scissors can be effectively used to divide the sample.) The ketone-soluble portions of the samples are dissolved as indicated for the making of standard rubber solutions on page 10, but the samples are transferred to 100-ml. volumetric flasks and made up to volume. The balance of the procedure is that outlined on pages 4 and 6. The results are expressed on the basis of the original weight of the crude-rubber product.

SUMMARY

Rapid semimicrophotometric methods for the determination of rubber and resins in small samples of guayule tissue are described. They may also be adapted for other rubber- and resins-bearing plants. The procedure has also been adapted for the determination of rubber in crude-rubber products and may be adapted for the analysis of synthetic rubbers.

The methods are based on the following principles: (1) Grinding the properly dried tissues or cutting the product to the required degree of fineness to permit quantitative extraction of rubber or resins; (2) using oxygenated solvents (ketones, acetates, and so forth for rubber; and acetonylacetone, cellosolve, and so forth for resins) with relatively high boiling points (115.8° to 230.7° C.), alone or in mixture, that permit the rapid extraction of rubber or resins in 20 to 30 minutes at relatively high temperatures (115° to 120°); (3) precipitating the rubber or resins from aliquots of the solutions by means of suitable precipitants (acidified ethanol for rubber and acidified water for resins); (4) making photometric readings of percentage of light transmittance after rubber or resins precipitation at a wave length that eliminates the error due to extraneous color (the determination relying on the development of a uniform turbidity rather than a color for purposes of analysis); (5) securing this uniform turbidity, or required degree of precision, by making the photometric readings at standing times (during which the readings are relatively stable) that are based on the concentrations of rubber or resins precipitated.

The steps in the procedure are as follows: (1) 0.2-gm. samples of finely ground, or divided, and dried material are extracted with 20 ml. of the solvent in a constant-temperature oil bath at 115° C., for 30 minutes. (2) After being cooled, the extracts are made up to a 25-ml. volume, are clarified by centrifuging, and three 5-ml. aliquots of the clarified extract are transferred to 25-ml. volumetric flasks. (3) For a trial reading one of these is first made up to volume with the precipitant; after it has stood for 4 minutes, photometric readings of percentage of light transmittance on a spectrophotometer at wave length of 750 mμ or on a colorimeter provided with the proper monochromatic filter combination for selecting approximately wave length 750 mμ are made. The readings obtained for the 4-minute trial read-

ings are referred to a table of equivalents previously prepared on the basis of 4-minute readings for the range of concentrations of rubber or resins to be encountered, and giving the standing time for rubber or resins after precipitation before photometric readings are made. The remaining duplicate aliquots are then made up to volume and readings are made at the standing time indicated in the table for the final readings. (4) The milligrams or percentages of rubber or resins present are read from a prepared table of equivalents for the photometric readings (based in each case on experimental data and using as standards pure rubber in rubber analysis and guayule resins exudate in resins determination).

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